IMMUNOLOGIAL PROFILE IN CHILDHOOD TUBERCULOSIS

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C E R T I F I C A T E

This is to certify that the work entitled "IMMUNOLOGICAL PROFILE IN CHILDHOOD TUBERCULOSIS" has been conducted by AJAY SOOD in the Department of Paediatrics, M.L.B. Medical College, Jhansi.

He has put in the necessary stay in the department as per the rules and regulations of Bundelkhand University, Jhansi.

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CERTIFICATE

This is to certify that the work entitled "IMMUNOLOGICAL PROFILE IN CHILDHOOD TUBERCULOSIS" has been conducted by AJAY SOOD, under my guidance and supervision in the Department of Paediatrics, M.L.B. Medical College, Jhansi.

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Ajay Sood

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C O N T E N T S

INTRODUCTION

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Tuberculosis is a necrotising bacterial infection with protean manifestations and wide distribution. The lungs are most commonly affected, but lesions may also occur in the kidneys, bone, lymphnodes and meninges or disseminated throughout the body. Infection may cause clinical disease either shortly after inoculation or after a period of months or decades of dormancy.

Morbidity and mortality due to tuberculosis are significant problem in developing countries not sparing children. Tuberculosis ranks among the five leading causes of morbidity and mortality in India. Incidence of tuberculosis in children (below 14 years) has been reported 2.7% in the general population and 3.4% in all patients registered in hospital (Manchanda et al, 1966). Incidence is found as high as 6.6% of total admission in the hospital. Different studies done by various workers have shown that 24% of children below 6 years, 40% of those below 12 years, had already contracted the infection as indicated by positive tuberculin reaction.

Tuberculosis is a major health problem in the developing countries. On the basis of criterion laid down by W.H.O., no single country in the world has succeeded in

reaching the point of control. Prevalence of infection in the age group of 0 - 14 years in developed countries is about 2 to 3% as compared to the prevalence in developing countries which is about 60% to 80%.

Tuberculosis in infancy and childhood posses serious problem particularly because of its high morbidity and mortality. Vague symptomatology and dreaded complication like meningeal, milliary and disseminated tuberculosis are common. Therefore, it deserves special emphasis and its early diagnosis and prompt treatment is very important.

Definitive diagnosis of tuberculosis can be made only by demonstrating acid fast bacilli in the appropriate material. This however, is achieved in only a small proportion of children with tuberculosis. So more often the diagnosis of tuberculosis is presumptive based on a history of contact, clinical findings, haematological examination and skiagram chest and positive mantoux test.

History of contact with an infectious patient of tuberculosis is often not available and at times deliberately defined due to social stigma of the disease. Clinical features at the onset are vague resulting in delay in diagnosis. Haematological changes in the form of decreased haemoglobin, lymphocytosis and raised erythrocytes sedimentation rate are non-specific as these changes can

take place in any chronic illness. Radiological changes in the lungs are only suggestive and not very confirmatory. In tubercular meningitis cytological and biochemical changes in cerebrospinal fluid may be absent in some cases, while similar alternation may occur in viral and partially treated tuberculosis.

Great reliance is therefore placed upon a positive mantoux reaction to distinguish tubercular from non-tubercular infection. This has proved partially useful in West, where a positive mantoux reaction is reported in 90% of patients of tuberculosis. But in India, positive mantoux reaction is recorded in only 30 - 40% of cases. This high incidence of this tubercular angery may be due to malnutrition as demonstrated by Harland in East Africa or due to immuno-deficiency which may be primary or secondary. Therefore, this test once considered to be an important screening test is losing its significance in our country because it may leave many cases of tuberculosis undetected.

In recent years, there has been increasing documentation about the value of BCG vaccination as a diagnostic trial. It is believed to be far superior to tuberculin test, because it is very sensitive and reliable test and it is generally positive even in situations like severe malnutrition, milliary tuberculosis and tubercular meningitis.

Cell mediated immunity is responsible for protection against tuberculosis. Tubercular hypersensitivity is an essential part of cell mediated immunity even though most of the patients produce humoral response against various antigens of tubercular bacilli, they are not protective. Bhatnagar et al (1977) have demonstrated an inverse relation between humoral and cellular response in tuberculosis.

Man has no inherited immunity against tuberculosis. It is acquired as a result of natural infection or B.C.G. vaccination. Past infection with atypical mycobacteria is also credited with certain amount of naturally acquired immunity. Antibodies, are produced as a resistance to infection. Both delayed hypersensitivity and acquired resistance to tuberculous are cell mediated response. It proves adequate to limit further multiplication and spread of bacilli.

Umpteen worker in the past have demonstrated that cell mediated immunity is compromised in different forms of tuberculous there being a direct correlation of the depression of cell mediated response to the severity and dissemination of tuberculosis.

With this prospective in mind, this study was planned with the following aims and objectives.

AIMS AND OBJECTIVES

1. To study the delayed hyper-sensitivity response in all forms of tuberculosis using B.C.G. and Mantoux test.

2. Study of T and B lymphocytes in conjugation with delayed hyper-sensitivity in the cases of different types of tuberculosis.

3. To evaluate a relationship between childhood tuberculosis and malnutrition vis-a-vis immunological profile.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

Tuberculosis is a very common disease in developing countries and involves different organs of the body. Tuberculous infection remains dominant in majority of the infected adults though it may become active at any time. However, the differences between infection and disease is narrow in children.

An apparent silent infection may result in a dangerous disease like milliary, meningeal or disseminated tuberculosis in an immunocompromised child (Udani PM, 1983 and Seth V, Nath, Singh, 1985).

According to an ICMR survey, the incidence of tuberculosis in India is about 1 in 50.

Understanding of the fundamental importance of the first or primary infection came slowly over the past century and the concept of primary infection evolved from studies of Parrot (1876), Gohn (1912), Ranke (1917) and Wallgren (1938). Yet even now the significance of primary infection for the continuance of tuberculous infection from one generation to next generation is not sufficiently recognized.

Nomenclature and History

Tuberculosis was named to indicate its formation of firm nodules or tubercles. For many years chronic form (often called phthisis or consumption) was considered a degenerative or hereditary disease quite unrelated to tuberculosis of childhood which was obvious by infections. The ancient writings of Indian medicine indicate that tuberculosis was existing in India more than 2000 years ago. Hippocrates (460 BC) the father of medicine, called it phthisis which means to dry up. The disease was also referred to as "captain of the men of death" and great consumption was considered. Lalnnee (1819) was the first to recognize the chronic form as merely a later development in the same infection. Koch (1882) identified the causative organism. Von Pirguit (1907) discovered the tuberculin test. Soon after First World War, BCG vaccine evolved by the French scientists, Calmette and Guerin, was tested in 1921. In India, BCG vaccine was introduced in 1949. Some human races (Caucasian, Mongolian) have lived with tubercle bacilli throughout much of their history and in them the infection produces a more chronic disease only rarely being fulminant. On the other hand, African, American Indians and Eskimo peoples have had contact with tuberculin over a much shorter period and in them the infection is more peone to produce fulminant disease. The death rate from tuberculosis began to fall by 1900 in developing countries.

For the person with clinical tuberculosis, however, the most important development occured in 1944 with discovery of streptomycin and later on other drugs.

Magnitude of the problem

Childhood tuberculosis is a public health problem of major importance in almost all countries. Tuberculosis is a universal disease as there is no single country in world which has succeeded in reaching the point of control that is "less than 1 percent tuberculin positivity among children in the age group 0 - 14 years - a criteria laid down by World Health Organization.

In India, tuberculosis infection is widespread in the country and on an average 50 percent of the population are infected at any one time. Morbidity and mortality due to tuberculin are significant problems in developing countries not sparing children (Udani et al, 1976). The number of deaths from tuberculosis in India is often quoted as 5,00,000 each year. Gordon et al (1961) reported a death rate of 110 per 1,00,000 population in the Punjab. Bordia (1968) estimated that about 80 to 100 persons die in India every year in a population of 1,00,000.

A survey done by Indian Council of Medical Research show the prevalence rate of active and probably active tuberculosis cases varied from 1.3 to 2.5 percent in

different zone and the bacteriologically positive case varied between 0.2 to 0.8 percent of population. It mean on an average 1.8 percent of the population have tuberculosis and 0.4 percent of them are infectious cases. It was also confirmed by Raj Narain et al (1963). The prevalence rate in the cities, towns and villages were nearly of same order. The prevalence of active disease in the population is 15 - 25 per 1,000 population. Of the 15 - 20 million active cases of tuberculosis, 4 to 5 million are bacillary. With such a vast magnitude of problem of infectious adult. The prevalence of infection in the child population is naturally very high. 40% of the children by the age of 6 years and nearly 80% by the age of 15 years can be considered as infected if 6 mm induration is considered as the cut-off point (Udani et al, 1982). Gothi et al (1979) have revealed that increasing the size of induration from 10-12 mm made only a difference of 2% in positive tuberculin rate.

With an annual rate of first infection, in children as 4% in India (Raj Narain, 1980), 3.64 million children in the age group of 0 - 4 years are infected annually (Udani, 1963). The problem of neuro-tuberculosis mainly tubercular meningitis in children has been highlighted by Udani (1983) on the basis of hospital data. Rao et al (1982) have shown that the risk of deaths in tubercular meningitis is 3 times more than in intra-thoracic tuberculosis in

children under 5 years and 1.5 times more in the age group of 5 - 14 years.

Age relation to Tuberculosis :

The prevalence of infection is about 40% in all the age group. With vast magnitude of the problem of infections in adult, the prevalence of infection in the child population is naturally very high. 40% of the children by the age of 6 years and 80% by the age of 15 years can be considered as infected if 6 mm induration is considered as cut-off point (Udani et al, 1982). Magotera et al (1974) described that at the age of 5-7 years, age incidence is higher and it is about 38.3%. It is followed by 2-5 years age group where it is about 25%.

Udani (1963) showed that 3.64 million children in the age group of 0-4 years are infected annually, with an annual rate of fresh infections in children as 4% in India.

Ramchandran et al (1968) done field studies conducted at Madras and showed that 24 percent of children below six years and 40 percent of those below 12 years had already contracted the infection as indicated by positive tuberculin reaction. Anergy demonstrated by false negative tuberculosis reaction in children suffering from tuberculosis poses a problem for diagnosis, in 50 percent of autopsy proven malnourished patients of tuberculosis, there was negative tuberculin reaction (Udani et al, 1976). In the

United States, it was the 8th leading cause of death by 1920 for children 1-4 years of age and by 1960 it was not one of the top 10 causes of death for any age group of children. This is in stark control to the persistant high incidence (one of the top 10 diseases in children) today in developing countries. Of new tuberculous infections revealed by conversion of tuberculin reaction from negative to positive, 5 to 15 percent progress to serious disease within 5 years if left untreated. The risk of direct progression varies with age. It is greatest when infection begins in the first years of life and next greatest in young adult and adolescents. Among those remaining well for 5 years, a further 3 to 5 percent may develop late recrudesance at some time during life.

A) Pathogenesis :

Initial infection : The tubercle bacillus may enter the body by way of the genito-urinary tract, the conjunctiva, the skin, the alimentary canal and the respiratory tract.

For practical purposes, this only route that is of importance in the United State is the lung, because they produce no toxin and no tissue reaction they remain free to multiply without deterrence (Harrison, 1980). After phagocytosis in the non-immune host, they remain viable within macrophages for an extended period. The organism

reach regional (hilar) nodes and even the blood stream before their progress is inhibited by the gradual development of specific immunity over a period of several weeks. At this time, the characteristic tissue reaction develops with epithileid cell granulomas and cessation necrosis in the pulmonary region, regional lymphnodes and any site to which bacilli has spread.

B) Silent Dissemination :

Early in the course of a new infection tubercle bacilli react the general circulation in varying numbers. This stage is important in the pathogenesis of tuberculosis because it is the time when bacilli react distant sites to establish metastatic foci of infection.

Latent infection :

When a tuberculous lesion regress and heals the infection enters a latent phase in which it may persist without producing illness. It may develop into clinical tuberculosis at any time if the persisting intra-cellular organisms began to multiply rapidly.

TUBERCULIN TEST

Historical :

Tuberculin was first introduced by Robert Koch in 1890. He announced the discovery of a harmless substance

which would both prevent and cure tuberculosis. He gave no clue as to the origin, nature or content of this magic stuff.

In 1907, Von Pirquet described a skin test which could be used in clinical work for the detection of those sensitised to tuberculin. He was the first worker who introduced the term 'allergy' to explain the altered reaction in form of cutaneous reaction.

Tuberculin preparations in use :

(a) Old tuberculin (OT)

(b) Purified tuberculin (PT)

(c) Purified protein derivative (PPD)

(a) Old tuberculin : Old tuberculin was originally prepared by Robert Koch in 1891. This is a crude preparation containing polysaccharides, nucleic acid and other soluble bacterial products as impurities. Some of the proteins of old tuberculin may have been denatured by heat and there is a variation in potency of different batches of it. Old tuberculin is not stable more than few weeks.

(b) Purified tuberculin : As studies of tuberculin test were conducted on a large scale, the need for a relative standard tuberculin product became more and more apparent.

In 1932, Seibert reported the preparation of a more purified tuberculin made by protein precipitation with trichlor acetic acid.

(c) <u>Purified protein derivative</u> : In 1934 purified protein derivative was prepared. This substance has smaller tuberculin protein molecules (Seibert, 1934 and Long et al, 1935).

P.P.D. now most widely used tuberculin, contains no protein other than that of tubercle bacilli. Its antigenicity is reduced by heating. P.P.D. solution is stabilised with a substance known as 'Tween'.

Old tuberculin has also been standardised by W.H.O. and both preparations are used extensively (Miller et al, 1963). Since human and bovine types of tubercle bacilli have a common allergen, the use of tuberculin from human tubercle bacilli is considered adequate to detect infection with either bacillus.

Since some of the proteins of O.T. and P.P.D. may have been denatured by heat. It is conceivable that these do not contain the full component of tuberculous proteins which is synthesised by the bacillus (Tripathi, 1972).

<u>Tuberculin tests in current use</u> :

In tuberculin testing either O.T. or P.P.D. is injected into the skin. There are several methods of tuberculin testing.

(a) Von-Pirquet test : It is qualitative and not quantitative test. The incidence of response to it, is atleast 10% less than to the intradermal method. It is painful especially when applied to the children, hence has largely fallen into disuse (Willis and Cummings, 1952).

The patch test is no longer regarded as reliable (Willis and Cummings, 1952). It is very satisfactory in young children, provided it is carefully performed and correctly read (Miller et al, 1963).

Dick (1950) advised that this should not be used in young children as it produce severe reactions.

(b) Multiple puncture test : This is widely used specially when large number of children are to be tested rapidly.

(c) Jet injection : This method uses a jet gun to deliver 5 TU of P.P.D. intradermally under high pressure.

(d) Mantoux test : It is most common method used for tuberculin testing. It is an intradermal test introduced by Mantoux in 1908. This test is performed by injecting 0.1 ml of standard solution of tuberculin (O.T. or P.P.D.) intradermally by a special tuberculin syringe with a short sharply bevelled 26 or 27 gauge 1/4" long needle into the cleansed skin of the flexor aspect of the left forearm.

The mantoux test has the advantage of accurate measurement and concentration of dose. It yields a higher

incidence of the reaction than Von-Pirquet test and has been found to be superior to Patch and Jelly tests (Willis and Cummings, 1952). When the quick result is necessary, the Mantoux test is more satisfactory (Miller et al, 1963).

Reading of Mantoux test

According to the diagnostic standards and classification of tuberculosis, American Lung Association, (1969), New York, the Mantoux test should be read 48-72 hours after injection. The reading should be made in good light with the forearm slightly flexed. Reactions are classified on the basis of induration (not erythema), which may be determined by inspection from a side view against the light as well as by direct light and by palpation with gentle stroking of the area with fingers. Reaction should be measured and recorded in millimeters as a largest diameter of induration at right angle to the long axis of the arm.

The correct time to read Mantoux reaction is on 3rd day (Mande, 1954), while Ustvedt (1951) and Greip and Bleiker (1957) suggested that it should be read on 3rd or 4th day.

Interpretation of Mantoux test :

In the past it was necessary to regard all reactions with induration of 5 mm or more in diameter as positive, without attaching significance to the size of reaction above

this minimal limit. Recent studies have shown that persons with good size reaction to 5 TU test (10 mm or more induration), are more likely to have tuberculosis than are the persons with smaller (5 to 9 mm) reactions (Diagnostic standard '1961' of the National Tuberculosis Association, America).

The following interpretations are recommended according to the Diagnostic standard and classification of Tuberculosis, American Lung Association, New York (1969).

(i) Positive Reaction (10 mm or more of induration) :-

This is a positive tuberculin test and almost always reflects sensitivity resulting from infection with M. Tuberculosis. The test does not need to be repeated for confirmation.

(ii) Doubtful reaction (5 - 9 mm of induration) :-

This doubtful reaction reflects sensitivity which can result from infection with either atypical mycobacteria or M. tuberculosis with incompletely developed sensitivity. If P.P.D. antigen for atypical mycobacteria are available, intradermal test with such antigens should be applied at the same time. If this person is known to have been in close contact with a case of proven active tuberculosis or has X-ray and clinical evidence of disease comparable with tuberculosis, he should be managed in the same fashion as a reactor with 10 mm. or more of induration.

(iii) Negative Reaction (4 mm and below of induration) :-

This reflects either a lack of tuberculin sensitivity or a low grade sensitivity which most likely is not due to M. tuberculosis infection. No repeat test is necessary unless there are other suggestive clinical evidence of tuberculosis.

Significance of a positive Mantoux test :

A positive Mantoux test is evidence that the person has been infected with tubercle bacillus and is allergic or hypersensitive to its proteins. The presence or absence of activity of a current lesion cannot be detected from the extent of reaction. Properly used, the test is more reliable method for detection of children who have had infection with the tubercle bacillus and it need further examination to determine whether the lesion is active or quiescent.

The severity of positive Mantoux reaction are categorised (Jaiswal, 1976) as below :

(i) Mild reaction	:	10 - 14 mm induration
(ii) Moderate "	:	15 - 30 mm induration
(iii) Severe "	:	Above 30 mm induration and/or ulceration, vesiculation and necrosis.

<u>Factors effecting Mantoux reaction</u> :

Under certain circumstances a child with tuberculous infection may have a negative reaction to tuberculin. These are :

(i) When the test is done during incubation period of the disease (Robert, 1975).

(ii) During advanced and terminal stages of tuberculosis when hypersensitivity to tuberculin is suppressed (Master, 1955).

(iii) Severe Malnutrition (Marland, 1965).

(iv) Administration of corticosteroids or immuno-suppressive agents (Salmon and Angel, 1961).

(v) Acute febrile illness (Robert, 1975).

(vi) Certain exanthema e.g. Measles, Chicken-pox and even after injection of live measles vaccine and possibly after smallpox vaccine (Aronson, 1951 & Calwell, 1957).

(vii) Sarcoidosis, Leukaemia, Cirrhosis of liver, influenza (Master, 1955), Scarlet fever, Pertussis, Diphtheria and glandular fever (Calwell, 1957).

(viii) Desensitization by large dose of tuberculin or recurrent small doses of tuberculin and B.C.G. vaccination (Master, 1955).

(ix) Faulty technique or faulty tuberculin, as it deteriorates rapidly in warm climate (Achar & Vishwanathan, 1972).

(x) Severe dehydration (Robert, 1975).

(xi) Agammaglobulinaemia.

False positive reaction may occur in the cases infected with atypical mycobacteria (Freidman and Silverman, 1952).

Fallacies of Mantoux test :

In children less than 4 years of age, reaction of 10 mm or more is correlated with an active primary infection with a high risk of developing meningitis or milliary tuberculosis (Lincoln et al, 1960). The doubtful reaction (5-9 mm) could be due to infection with atypical mycobacteria (Bogen, 1960 and Narain et al, 1972) and BCG vaccination at birth (Stegen et al, 1969). Hence, reaction of 10 mm or more to 5 TU PPD are considered to indicate active infection in children less than 3 years and are recommended for INH prophylaxis by American Thoracic Society (1965). In India, 1 TU PPD R.T.-23 with tween 80 is used. It has been shown that the size of reaction to 1 TU PPD RT-23 with tween 80 and 5 TU of PPD RT-19-20-21-22 without tween 80 did not differ appreciably in person who are infected with virulent tubercle bacilli (Barua, 1964).

Evaluating the tuberculin test in a population of 60,000 in India using 1 TU of PPD RT-23 with tween 80 (Narain, 1973) suggested that in paediatric practice a reaction of 15 mm or more may be regarded as evidence of active disease.

Though a positive test has been reported from 22% (Smith and Vollum, 1954) to 85% (Lincoln, 1947) of cases of tuberculous meningitis, a negative reaction is not uncommon.

Rajnarain (1968) has shown that a repeat tuberculin test given at different sites may give significantly larger reaction than the initial test in absence of new infection.

The value of tuberculin test is graded when its limitations are well understood. It is important that the test should be done properly, as sub-cutaneous injection of tuberculin invariably results in a negative test.

<u>B.C.G. Vaccination</u> :

In the last 15 years B.C.G. vaccine had been used as a test for tuberculosis by some workers. In a non-tuberculous child, after BCG there is no reaction for 2-3 weeks, then a pappule slowly forms with an induration which develops into a pustular stage in 6-8 weeks, while a scar in 3 months. In some children, instead of a scar, a nodule is formed. In a child with tuberculous infection, B.C.G. is followed by the formation of a papule with induration within 24-48 hours, a pustule by 5-7 days and a

scab by 10-12 days. The acceleration of this BCG reaction occurs even in malnourished children. Moreover, direct BCG does not produce any local focal or general adverse reaction even in frank tuberculous cases. Direct B.C.G. is more sensitive and reliable test than mantoux and is of great advantage in developing countries not only in diagnosis but also in mass immunization (Udani et al, 1971).

B.C.G. test is considered successful if it produces a positive tuberculin reaction (5 mm or above) in a previously negative child with standard dose of B.C.G. (Miller, Seal and Taylor, 1963). Rajnarain and co-workers have shown that a number of persons fail to become tuberculin positive (Rajnarain, Nair, Ramnath Rao and Chandrashekar, 1966). In recommendations by W.H.O. (BCG Unit) regarding the tuberculin reaction, Dr. Carrol Palme introduced specific and non-specific sensitivity in human beings. An induration of less than 5 mm. was indication of non-specific immunity.

It can be concluded that BCG is more reliable and sensitive test in the diagnosis of tuberculosis than Mantoux test, particularly so in malnourished children. BCG injection in tuberculous children produced mild or moderate reactions in the majority of cases and a strong local reaction is rare. In none of these cases was any adverse effect to the course of tuberculous infection. Even in children with moderate or strongly positive mantoux test, B.C.G. injection did not produce severe or adverse reaction. In moribund cases,

both tuberculin and BCG tests may be negative. However, even here, B.C.G. is more valuable than mantoux test.

B.C.G. Vaccines :

The B.C.G. vaccines in current use are :

(a) Liquid vaccine,

(b) Freeze dried vaccine,

(c) Isoniazid resistant vaccine.

Freeze dried vaccine

Freeze dried B.C.G. vaccine is being used because of its great operational advantages; furthermore, it permits quality control when it is released. The vaccine is frozen and dried in the ampules, with sodium glutamate as the only additive, and with exact duplication of the gradients of temperature and drying that have been found best.

The major drawback to B.C.G. is the fact that it causes conversion of the tuberculin test.

Technique of vaccination :

Intradermal vaccination : This is the one, most widely accepted method in which B.C.G. vaccine is injected intradermally. This was initiated in Sweden, in 1927 (Wallgren, 1928). 0.1 ml liquid of freshly suspended solution of freeze dried B.C.G. vaccine is injected intradermally by a leak-proof tuberculin syringe, fitted with a short bevelled needle.

The vaccination site is skin of the left upper arm, just above the insertion of the deltoid muscle. The vaccine is injected to raise a wheal of 7-8 mm. size.

B.C.G. Reaction patterns :

Intradermal B.C.G. results in a local primary complex formation at the vaccination site, which heals spontaneously and is followed by skin-sensitivity.

0.1 ml. of vaccine will raise a whitish wheal of skin 7-8 mm. in size over which the hair follicles are observed as minor pits. If the wheal is smaller, say 3 mm. it indicates sub-cutaneous injection which must be avoided as it will lead to lymphadenopathy and abscess formation. The wheal settles down as the vaccine is absorbed in 20-30 minutes. By the 3rd or 4th week an indurated area of 5-8 mm with a central papule appears, which gradually increases in size and by the 6th week, it reaches its maximum size of 8-10 mm. Subsequently a crust forms on the surface which later gets detached, exposing a shallow ulcer of 5-6 mm size which heals spontaneously by 12th week, leaving a 5-7 mm round hypopigmented permanent scar on the skin.

The reaction pattern takes about 3 months to complete. 4-12 weeks after vaccination, allergy develops which can be demonstrated by a Mantoux conversion.

Accelerated reaction occurs in tuberculin positive, tuberculin negatives in pre-allergic phase of natural

infection or in previously B.C.G. vaccinated persons with "Infra-tuberculin allergy" i.e. a state of partial hyper-sensitivity in which sensitivity to tuberculin has faded, but allergy to bacillary proteins or body has persisted (Friedman and Silverman, 1952).

Various types of B.C.G. positive reaction pattern are as follows :

1. Severity of B.C.G. reaction : It was categorised as (Jaiswal, et al, 1976) :

   - Mild reaction: Papule with 5-10 mm. induration.
   - Moderate reaction : Papule with 11-20 mm. induration.
   - Severe reaction : Papule with more than 20 mm. induration.

2. Types of B.C.G. reaction : These were classified as (Udani et al, 1971) :

   - Classical reaction : If papule with induration above 5 mm. appeared within 24-48 hours followed by pustule in 3-5 days, ulcer by 7th day and scab in 10-15 days.
   - Accelerated reaction : If papule appeared with 6-12 hours pustule on 3rd day and scab by 7th day.
   - Delayed reaction : If papule appeared after 72 hours and later course of reaction was similar to classical type.

<u>Efficiency of B.C.G. Test in diagnosis of Tuberculosis in infancy and early childhood</u> :

These are following :

(i) B.C.G. test is more reliable and sensitive as compared to mantoux test (Udani et al, 1981; Desai et al, 1972). A negative test excludes tuberculosis better than negative tuberculin test (Udani et al, 1971; Prahraj et al, 1977).

(ii) More reliable even in malnourished children (Udani et al, 1971) and in moribund cases with advanced tuberculosis (Lothe et al, 1973).

(iii) A very sensitive test for screening cases in early stage of disease (Desai et al, 1972; Jaiswal et al, 1976).

(iv) B.C.G. test is positive more often as it is stronger antigenically, being equal to 10 TU of PPD (Chaudhary et al, 1974).

(v) Complications following B.C.G. test in tubercular subjects are negligible (Geser et al, 1966; Dixit et al, 1976).

(vi) B.C.G. test provides protein in natural form and thus results are more rapid and sensitive (Dixit et al. 1976).

(vii) In children having high fever and dehydration, B.C.G. test is more sensitive than tuberculin test (Jaiswal et al, 1976).

(viii) On comparison of diagnostic value of different methods used for diagnosis of tuberculosis, B.C.G. test is most effective method being positive in 90% of cases followed by X-ray chest in 81% (Jaiswal, 1976).

(ix) It serves a double purpose of prophylaxis and diagnosis, fact which has got a great epidemiological significance (Lothe et al, 1973).

(x) B.C.G. vaccination of persons already infected with tubercle bacilli does not cause any activation of pulmonary lesion nor increases the risk of onset of tuberculosis (Geser et al, 1966; Chaudhary et al, 1974).

(xi) When Mantoux test is doubtful (Friedman and Silverman, 1952).

(xii) Results are available in shorter time i.e. 24-48 hours (Griffith, 1959; Dixit et al, 1976).

(xiii) To find out the correct prevalence of tuberculosis, incidence of positive B.C.G. test will give better results than positive tuberculin test (Desai et al, 1972).

Immunologic System :

Immunologic system is the part of host defence. its primary function is to protect against invasion by infectious agent. The major cost of this protection are allergy, auto-immunity and rejection of organ transplant. There are four major limbs of immunologic system, T lymphocyte, B lymphocyte, phagocyte and complement.

T and B Cell lymphocyte

Harris et al (1945) had shown that the lymphocytes were involved in the immunological mechanism. It is now recognised that lymphocytes from an indispensable component or body immune status and embodies that precursor of cells that will give rise both cell mediated immunity and humoral immunity. Although peripheral blood lymphocytes accounts only 0.2% of total lymphoid tissue in human body (Osgoad, 1954), there is free and extensive migration of lymphocytes by blood and lymph between various lymphoid organ, connective tissue and bone marrow. This continuous intermingling of all variety of lymphocytes makes them a perfect vehicle for the transportation and dissemination of viruses, bacterias and other antigens, antibody information throughout the body (Yoffey, 1964).

T lymphocytes play a major role in immune response to facultative organisms, tissue or organ graft and certain infections with viruses, B lymphocytes mature to become antibody producing plasma cells and play a role in humoral

immunity response (Rowland, 1975) lymphocytes circulate 4 to 6 times a day. T cells accounts for as many as 70% of peripheral blood lymphocytes while 20-25% are B cells (Lukes et al, 1974).

T lymphocytes are grouped broadly into modulator cells, effector cells, and cell producing lymphokines. Modulator cells are further divided into two categories. Those that initinte (helper or inducer) and those that tends to terminate (supressor cells) immune response (Reinherz and Schlossaman, 1980). The production of antibody by B lymphocytes requires the participation of helper T cells. A possible mechanism for subsequent termination of antibody production is the activity of suppressor cells. There appears to be a sub-population of inducer T lymphocyte required to induce the function of the T suppressor lymphocyte (Morimoto et al, 1981). In addition to modulatory lymphocyte, there are the T lymphocyte called cytotoxic effector cells. These cells are able to recognise foreign or altered self antigen. on the surface of cell and to destroy the cells (Paul, W.E., 1980). The other function of T lymphocytes is secretion of lymphokines, these low molecular weight substance secreted by activated T lymphocytes, affect the function of other cells in the surrounding environment. T cells secretes one type of interferon, a lymphokine that stimulates other cells to develop anti-viral activity.

Macrophage migration inhibition factor secreted by stimulated T cells causes activation and immobilisation of macrophages at the site of an inflammatory response (Rocklin et al, 1980). Interleukin-2 is lymphokine that promotes activation and division of other T lymphocytes (Gillis, 1983).

Recent advances indicates that there are two types of cell mediated response to mycobacterium tuberculosis, both of which produces positive tuberculin reaction. They are referred to as the Listeria type and the Koch type response. Rook and Gaw et al (1981) showed that the listeria type of response correlate strongly with protection and is now considered to contribute to protection in man whereas the Koch type response is either irrelevant or antagonist to protection in man.

B Lymphocyte :

B lymphocytes synthesize and excreate specific antibodies and serve as receptors for antigen. Plasma cell represent the extreme form of B cell differentiation. Earlier studies suggested the B cells could be distinguished from T cell by recognition of finger link cytoplasmic processes which were more numerous on B cells. B cells show single antigenic specificity on their surface where exposed to the relevant antigen processed by a macrophage. Under the influence of signals from antigenic specific

T lymphocyte. B lymphocyte differents into plasma cells which secrete antibody of the same specificity as originally found in its progenator. Lymphocyte having surface IgM, IgG, IgA, IgE, IgD make up the greatest number of B cells in peripheral blood of adult. Nearly 15-30% of peripheral lymphocytes are identified as B lymphocyte. B cell can be shown to have cell surface receptors this interact specially with certain components of complements. Sheep red blood cells prepared under appropriate condition (EAC) from rosette with human B cells. Lymphocyte membrane possesses temperature sensitive, mobile cell surface components.

Immunoglobins which are widely dispersed over the cell surface form a "cap" when cells are exposed at 37°C temperature, to labelled antiserum (essential to produce cross linking of the surface Immunoglobulins). Capping may be effective in producing confirmational changes of all surface receptors permitting necessary redistribution of these important molecules. Such "capping" may be a part of the process of differentiation of lymphocytes to plasma cells (Graves et al, 1973). Adherence of lymphocyte and macrophage to the indicator system is known as EAC rosette. B cells are commonly identified by immunoglobulin on sig marker.

<u>Phagocytosis</u> :

Third component of immune system comprises of fixed phagocytosis of reticulo-endothelial system as well as

wandering phagocytes (polymorpho-nuclear or mononuclear myeloid cells).

The macrophage has two critical roles - first is that it initiate specific immune response. Second function of macrophage is that its products exert a modulatory function on inflammatory response. Enzyme inhibitor such as plasmin and alpha-2 macroglobulin blocks the action of proteolytic enzymes. Macrophages product, prostaglandin, has been incrominated in suppression of lymphocyte function in vitro (Rice et al, 1979).

T and B cell count :

T and B lymphocyte can be identified by various methods. However, B cell poses surface immunoglobulins detectable by direct immuno-fluorescence.

Variation of lymphocyte count with age and sex :

Zochoraki and co-worker (1971) noted that there was no significant variation of lymphocyte count with age and sex.

Wybran et al (1972) found that there was no difference in T and B cell count of infant and children. Wkesler and Hutteroth (1974) found no difference in total lymphocyte and relative number of T lymphocyte in the peripheral blood of young children and adult individuals.

In most of the studies regarding the deviation of T lymphocyte and B lymphocytes counts in disease. Age characteristic of the central data have not been given, though Elhileli and associates (1978) have emphasized the importance of using age matched control.

Normal distribution of T and B lymphocytes :

Fiesher et al (1975) studied the sub-population of T and B lymphocytes in the peripheral blood of children and adult using E and EAC rosette assays. Children under 18 months of age were found to have decreased percentage of E-binding (T) lymphocytes and an increased percentage of EAC binding (B) lymphocyte as compared to older children (18 months to 10 years) and adults. The absolute number of E-binding and EAC binding lymphocytes was increased in children under 18 months of age.

Neighburger et al (1976) studied the distribution of T and B lymphocytes in peripheral blood of children and adult. They found the following distribution :

	T cell %	B cell %
Children	44.0 ± 4.2	30.4 ± 3.1
Adult	46.3 ± 1.6	26.8 ± 2.3

	T (E Binding cells)			
	Percentage		Absolute number	
	Mean ± S.D.	Range	Mean ± S.D.	Range
< 18 months	50.2 ± 3.7	33 - 67	2970 ± 690	1620 - 4320
18 months - 10 yrs.	56.8 ± 5.9	45 - 69	1840 ± 640	59 - 3090
Adult	64.0 ± 6.9	51 - 78	1910 ± 590	750 - 3070
	B (EAC Binding) cells			
< 18 months	26.2 ± 6.3	14 - 39	1530 ± 540	470 - 2590
18 months - 10 yrs.	22.7 ± 3.4	16 - 29	720 ± 280	170 - 1270
Adult (> 10 yrs.)	17.2 ± 3.9	11 - 23	540 ± 170	170 - 510

IMMUNOLOGY OF TUBERCULOSIS

The immunology of tuberculosis is a subject which has attracted tremendous interest in recent years. Still there are many aspects which remain poorly understood. Tuberculosis remains the classic example of a disease that is controlled entirely by cell mediated immunity involving the macrophage as the effector cell and the lymphocyte, (especially the T-cell) as the immuno-responsive cell (Mackaness, 1968). This type of immunity is also called

acquired cellular resistance. Cell-mediated immunity is essentially a local phenomenon carried out by macrophages that are activated locally at the site of infection by lymphocytes and their lymphokines. It is intimately linked with cellular hypersensitivity.

The specific immune response leading to macrophage activation and to granuloma formation is cell mediated and cannot be passively transferred with humoral antibodies. Following stimulation by the appropriate antigen, thymus dependent lymphocytes (T-cells) proliferate and synthesize a number of hormone like molecules termed lymphokines. Adams (1982) revealed that the nature and proportion of the T-cell subsets involved in the immune response differ from pathogen to pathogen and the ultimate effect on the macrophage is not quite similar in each case. The T-cells do not themselves effect the cell-mediated antibacterial immunity; rather they act indirectly through lymphokines which act via macrophages. The lymphokines attract macrophages to the site of infection and activate them to kill bacteria that these cells ingest.

Crowle et al (1983) has summarized the following characteristics of tuberculo-immunity :

1. It is cell-mediated.

2. It is expressed by appropriately activated macrophages.

3. The macrophages are appropriately activated by an immune lymphokine.

4. The immune lymphokine is made by immune T-lymphocytes which are activated by immunising antigen of the tubercle bacillus.

<u>Macrophages</u> :

When tubercle bacilli infect an animal or man, they are readily phagocytosed first by the PMN cells in which they multiply. The killing of these organism is by macrophages, first described by Lurie (1943). Human tuberculo-immunity as summarized by Crowle et al (1983) has mechanisms similar to mouse tuberculo-immunity. The necessary human cells can be readily obtained and made to function analogously in vitro. Bergholtz and Thorsby (1979) and McCalmon and Kirkegaard et al (1980) have demonstrated specific production of lymphokines by incubating peripheral blood monocytes, T-lymphocytes drawn from a tuberculin positive individual and PPD. The bacteria continue to multiply slowly but regularly in macrophages that come to the site of infection where the PMN cells have failed. Some of the infected macrophages reach the draining lymphnodes where they present tubercle bacillus antigen to T-lymphocytes. Specifically responsive clones of T-cells become activated and replicate, then leave the lymph node in large numbers and circulate in the blood

throughout the body where they give the infected subject two hallmarks of tuberculosis infection :-

- Tuberculin hypersensitivity (T-cells reactive to tuberculo-proteins).

- Tuberculo-immunity (T-cells reactive to immunising antigen).

Dannenberg et al (1982), have shown that many macrophages are killed in the process of pathogen-macrophage interaction in tuberculosis and hence the ability of the host to overcome the infection depends on the speed and effectiveness of the activation of new macrophages entering the lesion.

T-cells :

Events following infection are dominated by antigen stimulating T-cells. Monocytes must present the antigen to the T-cell acting in a helper capacity. Suppressor T-cells and suppressor macrophages suppress the T-cell function. The balance between helper and suppressor effects of T-cells is fundamental in the immune system of tuberculosis.

Lymphokines :

There is very strong evidence that CMI in tuberculosis is due to the activation of macrophages by soluble factors (lymphokines) liberated by antigen-specific

helper T-cells. Rook (1983) postulated that these activated macrophages may be further stimulated to secrete a factor or factors that cause tissue necrosis by a second signal which would be a lymphokine from a distinct set of T-cells. This lymphokine might merely prime the macrophages to react in this way in response to bacterial products.

Various lymphokines are :-

(i) Macrophage Activation Factor (MAF) which induces a marked increase in the metabolic activity of the macrophages which in turn leads to enhanced phagocytosis and intra-cellular killing.

(ii) Migration Inhibition Factor (MIF).

(iii) Macrophage Fusion Factor (MFF) which contributes to the formation of the characteristic granuloma or tubercle, containing giant cells.

(iv) Growth Inhibition Factor (GIF).

Crowle et al (1983) have also put forward the hypothesis that the hypersensitivity T-cells probably are mainly destructive to the subject and produce GEF predominantly in addition to causing tissue damage and tubercle formation. The immune T-cells are mainly helpful inducers of tuberculo-static or tuberculo-cidal macrophages. All the site of infection, reactive T-cells respond to the infecting

tubercle bacilli and cause inflammation and macrophage accumulation. The immune lymphokine GIF activates the macrophages to kill ingested tubercle bacilli. The pathogenic lymphokine GEF may to a certain extent have a beneficial effect of stimulating bacillary replication and thus keep the bacilli susceptible to killing by activated macrophages. However, it will promote the growth of bacilli in caseous lesions where macrophages can not reach. Progression or regression of tuberculosis depends upon the balance between the production of predominantly GEF of GIF producing antigen responsive T-cells.

Tuberculin test and delayed hypersensitivity :

Though Tuberculin test is the most frequently performed clinical test, its immunological basis is poorly understood. Grange (1983) has rightly concluded that not only cell mediated immunity (CMI) and delayed hypersensitivity (DH) are separate phenomena but DH is antagonistic to protection if the reaction is excessive. Though DH and CMI are both induced by lymphocytes, these cells are divisible into subsets with quite different functions in response to different components of mycobacterial antigens. High levels of DH have an adverse effect on tuberculosis in children (Udani, 1982 b). The tuberculin test is usually read after 48 to 72 hours.

Grange (1983) has suggested that some patients develop an immediate or Type I reaction and this reaction may reduce the eventual 48 hour reaction because it assists the removal of antigen from the infection site by vaso-dilation. The second is Arthus or type III DH reaction which develops at 4 hours and reaches its maximum at 8 hours.

Delayed hypersensitivity in tuberculosis :

Delayed hypersensitivity is responsible for the rapid accumulation of lymphocytes and macrophages which become activated wherever tubercle bacilli and their tuberculin like antigens exist in the tissues. The reaction causes accelerated tubercle formation. This accelerated tubercle formation causes the destruction of inhaled tubercle bacilli that are not destroyed by alveolar macrophages.

Accumulation of a large number of macrophages and lymphocytes at the site of tuberculin injection is responsible for large tuberculin reaction and is a protective effort on the part of the body. A large tuberculin reaction has no prognostic value (Dannenberg, 1982).

Accelerated tubercle formation is the mechanism by which cell-mediated immunity is produced (Mackaness, 1968). It is the ineffective activation of blood borne macrophages which instead of killing organisms get themselves killed causing progression of disease.

Immune spectrum in Tuberculosis :

Lenzini (1977) described an immune spectrum with two polar forms, reactive and un-reactive tuberculosis (RR & UU). The reactive form (RR) is characterised by localised lesions with lymphocytes and epitheloid cells and by a marked early response to anti-tubercular drugs. Immunologically, this form shows evidence of active cell mediated immunity with little or no antibody response. In particular, the reaction of PPD is that of a typical delayed hypersensitivity response and is also reflected in the positive cellular response in vitro. The unreactive form (UU) is characterized by rapid diffusion of the lesions within the chest and to other organs and a poor response to treatment. This group shows immunologically a very poor or an absent cell mediated immune response, resulting in both Tuberculin test and Leucocyte Migration Inhibition Test (LMIT) being negative with abundant antibody response. In between these two polar forms is an intermediate reactive group (IR) showing characteristics of the two extreme polar groups RR and UU.

IMMUNOLOGICAL PROFILE IN CHILDHOOD TUBERCULOSIS

Bhatnagar et al (1977) studied the immunological profile in cases of tuberculosis. He studied cases of healed and active tuberculosis of varying severity and compared their immunological status (by T and B lymphocytes) with the values observed on his control group of cases. He observed that whereas in the control group of cases the T lymphocyte values were 65.0 $\pm$ 1.34%, on the other hand in cases of healed and active tuberculosis, this values

were 60 ± 2.45% and 56 ± 1.87% respectively. An important finding of this study was that the T lymphocyte was directly proportional to the severity of tuberculosis. Cases of milliary tuberculosis had lowest value of T lymphocyte count (38 ± 6.18%). He also observed that there was increase in B lymphocyte in all forms of tuberculosis. The workers were of the opinion that in tuberculosis, there is an inverse relation between humoral and cellular response and there is decreased immunity in tuberculosis and it decreases as severity of tuberculosis increases.

Vimlesh Seth et al (1981) were another worker to have studied the cell mediated immune response in childhood tuberculosis. They studied thirty children who were suffering from various types of tuberculosis in the age group of less than six years. In their clinical profile of tuberculosis, they had taken 14 cases of tubercular meningitis, 6 cases of primary complex, 3 cases of peritoneal tuberculosis with ascitis, 2 cases each of spinal tuberculosis and collapse, consolidation. One case each was taken of constrictive pericarditis with mediastinitis, cervical lymphodinitis and milliary tuberculosis respectively. Weight was recorded in all the children and they were classified into various grades of malnutrition according to the recommendation of Nutritional Sub-Committee of Indian Academy of Paediatrics, using 80

percent and above of the 50th percentile of weight. Investigation in the form of tuberculin test with 1 TU and 5 TU of PPD. X-ray of chest or any other involved part and cerebrospinal fluid examination cytology and biochemical investigations were done in each cases. They observed that a large proportion of the children were malnourished (86.7%). They observed that the tuberculin test with 5 TU (43.3%) was significantly higher ($P < 0.01$) than the positivity observed with 1 TU (13.3%). Regarding the T cell count, the workers observed that in all forms of tuberculosis, the average value of T cell percentage was decreased ($56 \pm 9.29\%$) as compared to their control value of $57.6 \pm 11.2\%$ though the values were not significant ($P > 0.05$). The worker had opinion that the marginal fall of T cell count was attributed to that it is because of afferent and efferent component of cell mediated immune response may not be significantly altered in childhood tuberculosis with associated malnutrition. Chernushenko et al (1981) studied 218 patients of adult type of tuberculosis in which 158 patients were freshly diagnosed tuberculosis and 60 patients were of chronic form. In the freshly diagnosed cases (150) they observed E rosette forming cells (T cells) to be significantly lower values ($23.7 \pm 1.1\%$) where compared to their control values of T cells which was 41.1 ± 1.8. The T cell count rose up to $33.9 \pm 1.3\%$ after treatment. They also observed 52 cases of focal tuberculosis which had $26.7 \pm 1.8\%$ E-rosette

forming cells, whereas in the control group of cases (20 cases), the values were 41.1 ± 1.8% and after treatment the count rose upto 37.6 ± 3.3%. They also studied the B lymphocyte count by complemental rosette formation and observed that the majority of patients, the B cell count did not differ much from the values observed in the control group of cases. B cell values were 12.2 ± 1.0 in different types of pulmonary tuberculosis where its value in the control group of cases were 15.6 ± 1.7%. They also observed that in patients with disseminated and cavernous tubercular lesion, B cell percentage were lower (10.9 ± 1.1 and 4.9 ± 2.3% respectively), whereas in control group, the B cell percentage was 15.0 ± 1.7%. From this observation, the workers made the conclusion that it is the deficiency of the T lymphocyte function which leads to the development of generalized forms of tuberculosis. They also concluded that the specific immunological shifts depend greatly both on the degree of sensitisation and the functional state of T and B lymphocyte.

Venketa Reddy et al (1985) studied the B cell count by EAC rosette formation in their series of 34 cases, having active tuberculosis and relapse cases of tuberculosis and also treated cases. They observed that in active cases of tuberculosis, the mean percentage of B cell were 32.4 ± 11.0 when compared to control value where it was 24.6 ± 8.7%. They were of opinion that EAC-rosette forming

cells in the peripheral blood of patients with pulmonary tuberculosis, active cases had significant increase in the level. So they concluded that B lymphocyte role in cell-mediated immunity though doubtful can not excluded.

Seth et al (1985) studied a series of 45 children with chronic cervical lymphodinitis of more than two weeks duration and investigated. Twenty normal children of same age group served as control. Only 18 cases were having tubercular lymphodinitis etiology based on the histo-pathological findings in lymphnodes subjected to opem biopsy. They observed that T cells were significantly lower ($P < 0.05$) in the tubercular group as compared to controls. T cell percentage was 33.2 ± 5.5 in tubercular lymphadenitis as compared to the control, whereas the values were $39 \pm 6.8\%$. They made the inference that the decrease in the number of T cells detected by early rosette formation could be due to the requistration of specifically reactive lymphnode. They also observed the B cell percentage in tubercular lymphadenitis were significantly low. B cell percentage were 13.94 ± 2.71 in tubercular lymphadenitis whereas B cell percentage in control group was of 19.84 ± 4.71.

Seth et al (1985) did another study which was designed to investigate the radiological spectrum and its correlation with the immune parameters in childhood

tuberculosis. In all the study group of cases, they performed the mantoux test and then correlated its result with different types of tuberculosis, having different grades of malnutrition. In all they studied 120 cases of different types of tuberculosis and observed that positive tuberculin reaction was 80% in cases of tubercular lymphadenitis, whereas it was only 72.8% in the cases of pulmonary tuberculosis. They also observed that in severe protein energy malnutrition positivity of tuberculin was very low. The values was only 37.5% whereas positivity in the cases of normal and under-nourished children were 68.8 and 62.3% respectively. They also tried to make a relation with various types of tuberculosis. They observed that the percentage of children having grade III malnutrition had very low incidence of mantoux positive without clinical manifestation (4%). However, pulmonary primary complex value was 6.7% and in tubercular lymphadenitis it was 8.1% where in milliary and meningeal tuberculosis, it was about 33.3% respectively. However, the workers studied the T cell count in the various forms of tuberculosis in which 25 are having only mantoux positive test without clinical manifestation but positive X-ray finding, 25 cases were having tubercular lymphadenitis and 70 cases were having progressive primary complex. T cell percentage was 58.93 ± 9.06, in progressive primary complex while in mantoux positive without clinical manifestation and tubercular lymphadenitis, T cell percentage was 62.6 ± 7.85

and 48.76 ± 10.42 respectively. So they observed that T cells was significantly lower in tubercular lymphadenitis group in comparison to progressive primary complex and mantoux positive without clinical manifestation. They were of opinion that in tubercular lymphadenitis and progressive primary complex there might be lesser number of a subject of immune competent T cells due to a low antigen load in well localised lesions of progressive primary complex and tubercular lymphadenitis. In mantoux positive without clinical manifestation, the host defence mechanisms attempt to localise the infection by preventing the migration of leucocytes by secreting differentially a larger amount of migration inhibitionary factor.

Seth et al (1987) studied a series of 135 cases of various types of childhood tuberculosis for estimation of B cell. They also studied the relationship of B.C.G. positivity, family history and nutritional status with tuberculosis. They observed that the family history was positive in 25 to 40 percentage of cases. They also observed that grade III malnutrition was maximum in tubercular meningitis followed by progressive primary disease where it was 15.0% and pulmonary primary complex where it was only 10.5%. They also observed that B.C.G. positivity was 40% each in the cases of tubercular meningitis and progressive primary disease while it was only 25% in the cases of pulmonary primary complex.

They observed that absolute lymphocyte count and percentage of B lymphocyte were also comparable amongst the three types of lesion (progressive primary disease, pulmonary primary complex and tubercular meningitis) and also when compared with the control group of 20 cases. However, they found that absolute B cell count were increased in the three types in comparison to control. The increase was maximum in pulmonary primary complex where it was 885.2 $\pm$ 391.6 followed by pulmonary primary disease (722.0 $\pm$ 679.8) and tubercular meningitis (679.3 $\pm$ 312.2) in comparison with control where the value was 662.7 $\pm$ 342.0. The B cell percentage in control was 19.8 $\pm$ 4.1 while in pulmonary primary disease it was 18.7 $\pm$ 5.2 while in progressive primary disease and tubercular meningitis the B cell percentages were 19.7 $\pm$ 5.6 and 18.9 $\pm$ 5.1 respectively.

Mathur et al (1989) studied a series of 20 children of less than 12 years of age having different types of tuberculosis and divided their cases into 3 groups on the basis of mantoux and BCG test. First group of 4 children was of control. Mantoux test negative but B.C.G. test was positive having negative mantoux test (induration less than 5 m.m.) and negative B.C.G. test after ruling out any abnormality in X-ray chest. Second group consisted of 4 children with positive mantoux response (induration 10 m.m. or more) and X-ray chest positive for tuberculosis. Last group was of 12 children who had negative mantoux reaction

but showed accelerated BCG response (induration of more than 5 m.m. after 48 hours) as well as radiological abnormalities in chest X-ray. All of twelve cases were evaluated to rule out the possibility of protein energy malnutrition using clinical and biochemical criteria. They observed the immunological profile in childhood tuberculosis by T cells. They done T lymphocyte count by E-rosette formation in the three groups. They observed that in controls, the mean number of cells forming E-rosettes was 67.25% while in Mantoux positive case it was 63.75%. Relatively fewer cells (44.3%) formed E-rosettes in the mantoux negative and B.C.G. positive cases of tuberculosis. This value was statistically significant ($P < 0.05$) when compared with controls and mantoux positive cases. They were the opinion that mantoux negative in the presence of active tuberculosis, in developing countries may be due to malnutrition and immuno-deficiency. They also made inference that T cell was significantly low because of immuno-deficiency.

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MATERIAL AND METHODS

MATERIAL AND METHODS

The study was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi, over the period of one year. Three hundred children suspected of having various types of tuberculosis in the age group of less than 12 years, attending Paediatrics Out door department, Well Baby Clinic and those admitted in Paediatrics ward were included in this study.

Selection of cases :

A total number of 300 cases clinically suspected of having tuberculosis were subjected to preliminary investigation of mantoux and B.C.G. test, and accordingly only mantoux and B.C.G. positive cases were taken up for the study. The cases were grouped as follows :

I) Control group : which included healthy children.

II) Study group : The study group comprised of -

a) Cases of Pulmonary tuberculosis : It included

i) Primary complex,

ii) Progressive primary complex,

iii) Consolidation.

b) Cases of cervical lymphadenitis.

c) Cases of tubercular meningitis.

d) Cases of milliary tuberculosis.

Selection of Controls :

These children were in the age group 0 to 12 years, were clinically healthy, not having any systemic illness and had not taken any corticosteroid or immuno-suppressive agent, and were not recently immunized. These children were having normal weight and none of these children had any evidence of tuberculosis. All these cases were mantoux and B.C.G. test negative and X-ray chest was also normal. Ten children were selected for control.

Selection of Study group :

In this group, all the children were symptomatic and showing evidence of different forms of childhood tuberculosis. These cases were either mantoux positive and or mantoux negative but BCG positive cases. Further confirmation of diagnosis of the different forms of tuberculosis was done clinically, radiologically and by doing a lumbar puncture. Accordingly, we had 20 cases of pulmonary tuberculosis, 15 cases of cervical lymphadenitis, 10 cases of tuberculosis meningitis and 5 cases of milliary tuberculosis.

Present, Past and Family History :

A detailed present, past and family history was taken in each case. An enquiry was made to elicit the history of tuberculosis in the family members, relatives or in the neighbourhood.

Immunization History :

History of vaccination mainly of B.C.G. was taken in every case.

Physical examination :

A thorough general and systemic examination was conducted in each case and were recorded on the pre-designed proforma. Due emphasis was given to record the anthropometric measurements in each case and the cases were then divided as those having normal weight for age and those having Protein Energy Malnutrition, according to the classification of Nutritional Sub-committee of Indian Academy of Paediatrics (1972). Accordingly, it was observed that out of 300 cases of tuberculosis examined, 125 had different grades of malnutrition.

Grades of malnutrition	Weight expressed in percentage of Harvard Standard (50th percentile)	No.of cases
Grade I	71 - 80%	36
Grade II	61 - 70%	42
Grade III	51 - 60%	32
Grade IV	$<$ 50%	15
Total No.of cases having malnutrition		125

Mantoux test :

In the mantoux test 1 TU of PPD, RT 23, with tween 80 as stabiliser, was injected and this was obtained from BCG vaccine laboratory, Madras, India. Storage of tuberculin was done in refrigerator at 2 - 4°C. The Volar surface of the left forearm was cleansed with rectified spirit. The clean skin was then held taut by squeezing and pressing upon the extensor surface of the forearm. A 1 ml. tuberculin syringe graduated upto 0.01 ml. well fitted with a number 26 gauge, short bevelled needle was used. 0.1 ml. of tuberculin was taken in the syringe for each dose and intradermal injection was given into the superficial layers of the skin, while the syringe was held almost parallel to the plane of the arm. Successful intradermal injection was considered by the raising up of a small, pale, wheal-like elevations of the skin about 6 - 10 mm. in diameter and resembling like mosquito bite. Care was taken to prevent subcutaneous injection.

Reading :- The reaction was recorded after 72 hours of injection. The forearm was slightly flexed and the induration at the site of testing was determined by inspection from a side view against the light, as well as by direct light. The indurated skin was palpated by the gentle stroking to confirm the findings of inspection with the forefinger. The largest transverse diameter of induration was recorded in mm. Eruthema surrounding the induration was

not taken into account. However, any other abnormality such as vesiculation, ulceration and necrosis was also recorded, if present.

Interpretation :

Cases with induration of 10 mm. or more were regarded as positive, 5 - 9 mm. as doubtful and 0 - 4 mm as negative reactors and categorised as group A, B and C respectively.

The conditions in which tuberculin reaction is suppressed due to temporary immuno-deficiency inspite of active tuberculous infection e.g. malnutrition, intercurrent infection, recent viral infections (e.g. measles, pertussis, chicken pox and mumps), recent vaccination with a viral vaccine (e.g. measles and possible small pox), drugs like corticosteroids and ACTH and high continued fever were kept in view and traced by examination of the children and careful history taken from the patients or parents.

In the present study all the children showing tuberculin reaction 10 mm. or more were considered as mantoux positive or positive reactors and categorised as group A. These cases were further investigated for tuberculosis.

All the children showing tuberculin reaction between 5 - 9 mm. were considered as doubtful reactors and

categorised as group B. These cases were left as such in the present study.

The negative tuberculin reactors were categorised as group C and BCG vaccination was given to these cases.

B.C.G. Vaccination and Test :

BCG vaccination was given to all the mantoux negative cases (with or without previous BCG scar). The fresh solution was made in distilled water and was utilized within 2 - 3 hours of reconstitution. Twenty dose ampules of freeze dried vaccine were reconstituted in 2 ml of distilled water just before use and was mixed thoroughly by shaking between the palms every time before injecting vaccine.

Technique : BCG test was done with single puncture technique by 1 ml. leak proof tuberculin syringe, graduated upto 0.01 ml, fitted with a number 26 gauge, short bevelled needle. The left upper arm just above the deltoid insertion was chosen as the site for vaccination. The skin overlying this area was cleansed with a spirit swab. Skin was made taut by holding the upper arm of the child. The freshly prepared vaccine was injected intradermally. A successful intradermal injection is indicated by the raising up of a whitish wheel of 7 - 8 mm. diameter over which the hair follicles were visualized as small pits. The cases were

advised not to take any drug for tuberculosis or corticosteroids before and after vaccination which might affect the response of BCG.

Reading was done after 48 hours of injection. The largest transverse diameter of induration was measured in mm (not erythema) by inspection and palpation in good light.

Interpretation :

Induration less than 5 mm. was regarded as usual response or negative BCG test, while induration of 5 mm. or more was regarded as accelerated positive BCG test. Cases showing usual response were considered as usual responders and categorised as group a. These cases were left as such in the present study. All cases showing positive BCG test were considered as accelerated responders and categorised as group b. All accelerated responders were subjected for further investigations of tuberculosis.

Investigations :

Positive tuberculin reactors and negative tuberculin reactors with accelerated BCG response were investigated on Koch's line thoroughly.

a) Blood Haemoglobin : It was estimated by Sahli's acid haematin method and expressed in gm/100 ml.

b) Total Leucocyte Count : It was done by using Neubaur Chamber expressed as cells/cubic millimeter.

c) Differential Leucocyte Count : It was estimated by using Leishmann's stain.

d) Absolute Lymphocyte Count : It was done by using Neubaur counting chamber and expressed as cells per cubic millimeter.

e) Erythrocyte Sedimentation Rate : It was done by using Wintrobe's method and expressed as mm. fall in first hour.

f) Skiagram chest (P.A. view) : It was done in all mantoux positive cases and in all mantoux negative cases showing accelerated BCG response.

g) Other specific investigation depending upon type of tuberculosis : It was done in those cases who were having tuberculosis other than pulmonary tuberculosis, for example, CSF examination in the cases of tubercular meningitis.

Diagnosis of tuberculosis was based on 4 - 5 criteria e.g. positive clinical history, evidence of disease on clinical examination, history of possible adult contact, positive radiological evidence, haematological investigations in favour like leucocytosis, lymphocytosis, decreased haemoglobin and raised ESR. On the basis of investigation, different types of tuberculosis was diagnosed. Control, mantoux positive and mantoux negative BCG positive cases having different types of tuberculosis were subjected for determination of cellular immunity in the form of T and B cell.

<u>Laboratory techniques</u> :

Material used -

- Heparin (Preservative free)
- Minimum essential medium (MEM) - Eagle
- Alsever's solution
- Phosphate buffer saline (PBS)
- Pooled normal human serum
- Anti-sheep haemolysin (Amboceptor)
- Methyline blue 0.2%.

Alsever's solution :

Glucose	:	24.6	gm
Trisodium citrate (dehydrate)	:	9.6	gm
Nacl	:	50.04	gm
Distilled water	:	1200	ml

pH of Alsever's solution was adjusted to 6.1 with 10% citric acid. Solution was sterilised by low pressure autoclaving and stored in a refrigerator.

Phosphate buffer saline (PBS) :

A) 0.15 M - Na_2 HPO_4 $2H_2O$ (23.4 gm/litre)

B) 0.15 M - Na_2 PO_4 21.3 gm/litre

C) Normal saline - 9.0 gm Nacl/litre.

For phosphate buffer saline (pH 7.4) solution A (18 ml) was mixed with solution B (82 ml) and then solution C (100 ml) was added. The solution was then sterilized by low pressure autoclaving and stored in a refrigerator.

<u>Pooled normal human serum</u> :- Venous blood was drawn asceptically into clean and dry test tubes 15 ml. each, from 4 persons. Test tubes were incubated in water bath at 37°C for 30 minutes and then at 40°C for 120 minutes. The clot from each tube was removed gently with a glass rod and the tubes were centrifuged. The clear serum from each of the tubes was collected and mixed with each other. Pooled serum was stored at -20°C. Small alliquotes were used only once after thawing.

<u>Collection of blood sample</u> :- 10 ml heparinised peripheral blood sample (25 unit of heparin/ml of blood) was collected in the sterile tube from each patient for T and B cell counts. Also the venous blood was simultaneously collected in double oxalate vial from the patient, for total and differential leukocyte counts.

<u>Total Leukocyte Count (TLC)</u> :- One in 20 dilution of blood was made by adding 0.02 ml blood to 0.38 ml of WBC diluting fluid (Turk's fluid) in 7.5 x 10 mm test tube. The suspension was mixed by gentle tilting and rotating by hand for 2 minutes. The Neubaur's counting chamber

was charged with suspension and viewed with 5 mm objective under a microscope. The number of leukocyte were counted and calculated as below :

$$TLC = N \times 200/cu\ mm.$$

'N' is number of leukocyte counted in each mm square area. Total four squares was counted, in which each large square contain 5 small square (total 80 small square will count).

Differential Leukocyte Count (DLC) :- A thin and uniformly prepared peripheral blood smear was stained for 8 to 10 minutes with Leishman stain washed with buffered solution (pH 6.8) containing $K\ H_2\ PO_4$, 9.19 m/1 & $Na_2\ HPO_4$, 9.5 m/1 (mixed together in the ratio of 1.03 : 1). The slide was then dried in air. Leukocytes were counted using oil emersion lens and the percent distribution of different leukocytes was calculated after counting 200 cells.

Absolute Lymphocyte Count (ALC) :- Absolute count was calculated in every case from the total and differential leukocyte count using the following formula -

$$ALC = \frac{TLC \times \%\ lymphocytes}{100}$$

Evaluation of T and B lymphocyte :

1. Preparation of lymphocyte rich plasma :

The lymphocytes were separated from the heparinised peripheral blood by gravity sedimentation method. 10 ml of

heparinised blood (25 unit/ml blood) collected in a sterilized test tube was kept up-right at room temperature for one hour. The leukocyte-rich plasma was collected and centrifuged at 1000 rpm for 15 minutes. The clear plasma was separated and the cell button was suspended in phosphate buffer saline PBS/MEM minimal essential medium. The concentration of lymphocyte suspension was adjusted to $2 - 3 \times 10^6$ per ml in PBS/MEM.

2. Preparation of sheep RBC solution :

Sheep blood collected in equal volume of Alsever's solution was stored in a refrigerator for 3 - 5 days and thereafter used upto 14 days. Sheep blood was washed thrice with buffer saline. One volume of packed cells was suspended in 18 volumes of buffer saline to give a slightly greater concentration than 5% suspension. One ml of this suspension was lysed with exactly 14 ml. of distilled water and optical density (OD) was measured at 540 ohm with distilled water as blank. A lysate with optical density of 0.7 represented 5% concentration or 1×10^9 cells/ml. From the O.D. of sample tested and volume of the suspension (vi). the final volume (vf) was calculated according to the relationship :

$$vf = \frac{vi \times O.D.}{0.7}$$

Finally suspension was adjusted to make a standard solution of sheep RBC.

<u>Demonstration of T cell by sheep RBC Rosette (E-Rosette)</u> :

Sheep RBC's were washed thrice with PBS and 0.5% suspension was made in M.E.M. solution. Lymphocyte count was adjusted to 2 - 3 x 10^6 per ml in PBS. To 0.5 ml of sheep RBC suspension was added 0.5 ml. of lymphocyte suspension in PBS and mixture was incubated for 15 minutes at 37^{o}C in water bath. After centrifugation for 5 minutes at 500 rpm, mixture was incubated at 4^{o}C over night. Supernatant was removed and pellet was resuspended in MEM (2-3 drops). Finally wet preparation was made and stained with methylene blue (0.2%) and 200 rosette forming cells were counted under a microscope to calculate the percentage of rosette forming cells.

When three or more SRBC's were seen adhering to a lymphocyte, it was considered as a rosette forming cell. The absolute T cell count was calculated as follows -

$$\text{Absolute T cell count} = \frac{\text{ALC x \% T cells}}{100}$$

<u>Demonstration of B cells by forming EAC rosette</u> (Fleisher et al. 1975 and Shevach et al. 1972) :

B lymphocytes in normal peripheral blood can be identified by the presence of at least three surface marker, receptors for modified components of complement, surface immunoglobulins and receptors for aggragated IgG.

Complement receptor bearing lymphocytes can be detected by the binding of antigen, Erythrocyte (E), antibody (A) and complement (C) to form EAC rosettes.

To 0.5 ml of 5% of SRBC suspension, 0.5 ml of anti-sheep haemolysin in appropriate dilution (1 : 400 assessed earlier) was added and incubated for 15 minutes at 37°C. After washing three times with phosphate buffer saline and resuspending in PBS and thereafter adding 0.5 ml of 1 : 10 diluted complement (Human serum), tube was incubated for 45 minutes at 37°C. The cells were washed with phosphate buffer saline and then resuspend to make a concentration of 0.5% of EAC in phosphate buffer saline.

To 0.5 ml suspension of lymphocyte (2-5 x 10^6 ml), 0.5 ml of EAC in PBS was added and incubated at 37°C for 30 minutes. The solution was resuspend and wet preparation was prepared and stained with 0.2% Methylene blue. Finally 200 cells were counted under the microscope to calculate the percentage of EAC rosette forming lymphocytes.

A group of three or more SRBC's adherent to a lymphocyte was considered as EAC rosette. Absolute B cell count was calculated as follows :

$$\text{Absolute B cell count} = \frac{\text{ALC x \% EAC rosettes}}{100}$$

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O B S E R V A T I O N S

OBSERVATIONS

The study was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi, over the period of one year. Three hundred children suspected of having various types of tuberculosis in the age group of less than 12 years, attending Paediatrics out door department, Well Baby Clinic and those admitted in Paediatrics ward were included in this study. A detailed history and clinical examination was done in every case and findings were recorded in a prepared proforma.

Presenting Features :

Important presenting features of suspected cases of tuberculosis are given in Table No. 1.

Table 1

Presenting features in cases selected for study.

Presenting features	No.	Percentage
Loss of appetite	102	34.00
Non-specific symptoms	134	44.66
Cough	76	25.33
Fever	97	32.33
Failure to thrive	124	41.33
Superficial lymphadenitis	242	80.66
Convulsion	39	13.00

Among 300 suspected cases, in 134 (44.66%) cases, the presenting features were non-specific or vague symptoms like occasional vomiting, irritability, not playful or not as active as before or not eating well.

Mantoux Test :

To all 300 suspected cases of tuberculosis, 0.1 ml of PPD was injected intradermally. Mantoux test was read after 72 hours and subsequently the cases were divided into 3 groups on the basis of induration. Results of Mantoux test are given in Table No. 2.

Table 2

Results of Mantoux test in suspected cases of tuberculosis.

Groups	Induration in m.m.	Mantoux Reaction	No.of cases	Percentage
A	10 or more	Positive	81	27.00
B	5 - 9 m.m.	Doubtful	80	26.67
C	0 - 4 m.m.	Negative	139	46.33
Total			300	100.00

Group A :- It comprised of 81 cases (27.00%) which were Mantoux positive. These cases were considered to be suffering from tuberculosis. Further diagnosis was confirmed by haematological investigation, radiology, cerebrospinal fluid examination and other specific examination.

Group B :- It comprised of 80 (26.67%) cases in which the reaction was doubtful.

Group C :- It comprised of 139 cases (46.33%) in which the reaction was negative.

BCG Vaccination : Mantoux negative cases (group C) were given B.C.G. vaccination and results were read after 48 hours of vaccination. The results of vaccination are given in Table No. 3.

Table 3

BCG Test in suspected cases of tuberculosis (Mantoux negative cases).

Groups	Induration in m.m.	Response	No.of cases	Percentage
a	Less than 5	Usual	69	49.64
b	5 or more	Accelerated	70	50.36
Total			139	100.00

Group a :- It comprised of 69 (49.64%) children as shown in Table No. 3. In these children, induration was less than 5 m.m. within 48 hours. These children was considered as usual responders.

Group b :- It comprised of 70 (50.36%) children in which induration was more than 5 m.m. after 48 hours of B.C.G. vaccination. These cases were considered to have an accelerated response to B.C.G. These children were subjected to further investigations to confirm the diagnosis and type of tuberculous infection.

B.C.G. Vaccination in Mantoux Doubtful Reaction :- B.C.G. vaccination was also given to those children who were having doubtful reaction (Group B in Table No. 2) to find out the positivity of B.C.G. vaccination in doubtful reactors. The results are given in Table No. 4.

Table 4

Results of B.C.G. Vaccination in Mantoux Doubtful Reaction children.

Groups	Induration in m.m.	Response	No.of cases	Percentage
a	Less than 5	Usual	27	33.75
b	5 or more	Accelerated	53	66.25
Total			80	100.00

Group a :- It comprised of 27 (33.75%) cases which showed the usual reaction. It means that these children did not show the B.C.G. test positive.

Group b :- It comprised of 53 (66.25%) cases which showed the accelerated or positive reaction. These children were investigated for tuberculosis. These children left as such in this study.

Mantoux Reaction in Malnourished children :

Mantoux test was done in children having different grades of malnutrition. The results are given in Table No. 5.

Table 5

Grade of malnutrition	No. of children	No. of children having positive reaction	Percentage
Grade I	36	26	72.22
Grade II	42	22	52.38
Grade III	32	8	25.00
Grade IV	15	3	20.00
Total	125	59	47.20

Table 5 shows that Mantoux positivity was maximum (72.22%) in the tubercular cases having grade I malnutrition and was minimum in cases of severe PEM, viz. in grade IV malnutrition. Mantoux positivity was only 20.00%.

Nutritional status in different types of tuberculosis :

Nutritional status was estimated in different types of tuberculosis irrespective of Mantoux positive and Mantoux negative BCG test positive. Results are given in Table No. 6.

Table 6

Type of lesion	No.of cases	Nutritional status			
		Normal	Grade I & II	Grade III	Grade IV
Primary complex	30	15 (50.0%)	15 (50.0%)	0	0
Progressive primary complex	21	6 (28.5%)	12 (57.0%)	2 (9.5%)	1 (4.8%)
Tubercular consolidation	22	7 (31.82%)	7 (31.82%)	5 (22.73%)	3 (13.63%)
Tubercular meningitis	32	4 (12.5%)	16 (50.0%)	6 (18.7%)	6 (18.7%)
Milliary tuberculosis	12	2 (16.7%)	6 (50.0%)	2 (16.7%)	2 (16.7%)
Cervical lymphadenitis	34	10 (29.4%)	18 (52.9%)	3 (8.8%)	3 (8.8%)
Total	151				

It is evident from Table No. 6 that there was a direct correlation between the severity of tuberculosis and the degree of PEM viz. dessiminated forms of tuberculosis had higher incidence and severity of malnutrition.

Investigation for Tuberculosis :

Mantoux positive as well as Mantoux negative but BCG positive cases were investigated further for tuberculosis.

Haematological Investigation :

Total leucocyte count was done in 151 cases (81 cases of group A (Mantoux positive) and 70 cases of sub-group b). Results of cases are given in Table No. 7.

Table 7

Total leucocyte count in suspected group of tuberculosis.

Groups	No.of cases	Leucocytosis		Normal leucocyte Count	
		No.	%	No.	%
I Mantoux positive	81	56	69.13	25	30.87
II Mantoux negative, BCG Positive	70	50	71.42	20	28.58
Total	151	106	70.19	45	29.81

Out of 151 cases, 106 cases (70.19%) showed leucocytosis while only 45 cases (29.81%) showed leucocyte within normal counts. It is evident that there was no significant difference in leucocyte count in Mantoux positive and in Mantoux negative B.C.G. positive cases.

Absolute lymphocyte count :

It was done in all the cases of the two suspected groups of tuberculosis. The results which were observed are given in Table No. 8.

Table 8

Absolute lymphocyte count in suspected groups of tuberculosis.

Groups	No.of cases	Absolute lymphocytosis		Normal lymphocyte count	
		No.	%	No.	%
Mantoux positive	81	49	60.47	32	39.53
Mantoux negative, BCG positive	70	42	60.00	28	40.00
Total	151	91	60.26	60	39.74

Out of 151 cases, 91 cases (60.26%) showed absolute lymphocytosis while 60 (39.74%) cases showed abnormal lymphocyte count within normal range.

In group A, out of 91 cases (60.47%) showed absolute lymphocytosis while 32 (39.53%) showed absolute lymphocyte within normal limits. In group B, out of 70 cases, 42 (60.00%) cases showed absolute lymphocytosis, while only 28 (40.00%) cases showed absolute lymphocyte count within normal limit.

Blood Haemoglobin :

It was estimated in 151 cases (81 cases of Group A and 70 cases of sub-group b). Results of Haemoglobin (Hb) estimated are given in Table No. 9.

Table 9

Hb level in suspected groups of tuberculosis.

Groups	No.of cases	Hb.less than 10 gm. No.	%	Hb.more than 10 gm. No.	%
Mantoux positive	81	50	61.72	31	38.28
Mantoux negative, BCG positive	70	51	72.85	19	27.15
Total	151	101	66.88	50	33.12

Out of 151 cases, 101 cases (66.88%) showed Hb level less than 10 gm% and 47 cases (33.12%) showed Hb level more than 10 gm%. In group A, 50 (61.72%) cases showed Hb less than 10 gm% while rest 30 (38.28%) cases showed Hb more than 10 gm%. In group b, 51 cases (72.85%) showed Hb less than 10 gm% while rest 19 cases (27.15%) showed Hb in normal range (more than 10 gm%). In group A Hb less than 10 gm were present in 67.28% cases while in sub-group b, it was present in 72.85% cases. In group A cases, Hb more than 10 gm was present in 38.28% cases while in sub-group b, it was present only in 32.72% cases.

Erythrocyte Sedimentation Rate :

It was estimated in 151 cases. Results of values of ESR is given in Table No. 10.

Table 10

ESR in suspected groups of tuberculosis.

Groups	No.of cases	Raised ESR		Normal ESR	
		No.	%	No.	%
Mantoux positive	81	61	75.30	20	24.70
Mantoux negative, BCG positive	70	47	67.14	23	32.86
Total	151	108	71.52	43	28.48

Results showed that out of 151 cases, ESR was raised in 108 cases (71.52%), while only 43 (28.78%) cases showed normal ESR. In group A, out of 81 cases, 61 cases (75.30%) showed raised ESR while 20 cases (24.70%) showed normal ESR. In group b, out of 70 cases, 47 (67.14%) cases showed raised ESR while only 23 cases (32.86%) showed normal ESR.

Cellular immunity in Mantoux positive and Mantoux negative BCG positive cases :

E-rosette Test : Rosette formation was considered when lymphocyte had 3 or more than 3 sheep RBCs attached to it. The results are shown in Table No. 11.

Table 11

T-lymphocyte count by E-rosette formation in mantoux positive and mantoux negative BCG positive cases and in control.

Groups	No.of cases	Range of T-cells %	T-lymphocyte percentage E-rosette count (Mean ± S.D.)
a) Control	10	59 - 70	60.8 ± 3.15
b) Mantoux positive	30	45 - 68	57.13 ± 6.43
c) Mantoux negative BCG positive	20	30 - 54	40.25 ± 5.47

$t_{a \times b} = 1.60$, $P > 0.05$,

$t_{a \times c} = 10.64$, $P < 0.001$.

$t_{b \times c} = 9.43$, $P < 0.001$

It is evident from table No. 11, that maximum depression of T cell count was observed in mantoux negative BCG positive cases and these values were statistically significant from the values observed in the control group of cases ($P < 0.001$). In mantoux positive cases, though the values of T cell count were less as compared to control values, no statistical significant difference was seen from the values observed in control group of cases. However, on further analysis, it was seen that there was clear difference and depression of T cell count between our 2 study groups (Mantoux positive and Mantoux negative BCG positive cases), values of T cell count being statistically decreased in the latter group ($P < 0.001$).

It is evident from table No. 12, that in mantoux positive cases, there was either normal nutrition or grade I and grade II malnutrition. It also shows that depression of T cells is directly related to malnutrition. As in normal nutrition, T cell is slightly higher than grade I and grade II malnutrition.

Table 12

Relation of T cell in different types of tuberculosis with malnutrition in Mantoux positive cases.

Type of tuberculosis	No.of cases	No.of cases having Mx.+ve	Malnutrition	No.	Range	T-cells % (mean)
Primary complex	10	9	Normal nutrition	4	62-68	64.6%
			Grade I malnutrition	3	58-64	60.3%
			Grade II malnutrition	1	47%	47%
Progressive primary complex	5	3	Normal nutrition	1	59%	59%
			Grade I	1	55%	55%
			Grade II	1	54%	54%
Tubercular consolidation	5	2	Grade I	1	64%	64%
			Grade II	1	60%	60%
Milliary tuberculosis	5	1	Grade II	1	54%	54%
Tubercular meningitis	10	3	Grade I	1	49%	49%
			Grade II	1	45%	45%
			Grade III	1	45%	45%
Cervical lymphadenitis	15	12	Normal nutrition	5	55-68	58.8%
			Grade I	4	52-66	57.5%
			Grade II	2	48-50	49%
			Grade III	1	49%	49%
Total	50	30				

Table 13

Relation of T cell in different types of tuberculosis with malnutrition in mantoux negative BCG positive cases.

Type of tuberculosis	No.of cases	No.of cases Mx. -ve BCG +ve	Malnutrition Type	No.	Range	T-cells %
Primary complex	10	1	Grade III	1	47%	47%
Progressive primary complex	5	2	Grade II	1	54%	54%
			Grade IV	1	39%	39%
Tubercular consolidation	5	3	Grade III	1	49%	49%
			Grade IV	2	36-39	37.5%
Milliary tuberculosis	5	4	Grade III	1	40%	40%
			Grade IV	3	30-39	35.7%
Tubercular meningitis	10	7	Grade III	3	39-42	40.3%
			Grade IV	4	33-39	36.5%
Cervical lymphadenitis	15	3	Grade II	1	46%	46%
			Grade III	2	44-46	45%
Total cases	50	20				

It is evident from table No. 13, that in severe cases of tuberculosis, malnutrition of grade III and grade IV is more common. Depression of T cells were more in the cases having severe types (grade III and grade IV) of malnutrition.

Table 14

T-cell percentage in different types of tuberculosis.

Type of tuberculosis	No.of cases	T-cell % (Mean $\pm$ S.D.)
a) Control	10	60.8 ± 3.15
b) Primary complex	10	60.2 ± 5.53
c) Progressive primary complex	5	52.2 ± 6.8
d) Tubercular consolidation	5	49.6 ± 11.07
e) Tubercular meningitis	10	39.9 ± 5.09
f) Milliary tuberculosis	5	46.4 ± 7.55
g) Cervical lymphadenitis	15	53.9 ± 6.85
Total number of cases	60	

$t_{ab} = 0.63$, $P > 0.05$

$t_{ac} = 3.12$, $P < 0.01$

$t_{ad} = 2.78$, $P < 0.05$

$t_{ae} = 10.48$, $P < 0.001$

$t_{af} = 4.83$, $P < 0.001$

$t_{ag} = 2.86$, $P < 0.01$

$t_{bc} = 2.57$, $P < 0.05$

$t_{bd} = 2.50$, $P < 0.05$

$t_{be} = 8.09$, $P < 0.001$

$t_{bf} = 3.74$, $P < 0.01$

$t_{bg} = 2.33$, $P < 0.05$

$t_{cd} = 0.4$, $P > 0.05$

$t_{ce} = 3.66$, $P < 0.01$

$t_{cf} = 1.14$, $P > 0.05$

$t_{cg} = 0.46$, $P > 0.05$

$t_{de} = 2.16$, $P > 0.05$

$t_{df} = 0.48$, $P > 0.05$

$t_{dg} = 0.97$, $P > 0.05$

$t_{ef} = 1.82$, $P > 0.05$

$t_{eg} = 5.30$, $P < 0.001$

$t_{fg} = 1.96$, $P > 0.05$

It is evident from table No. 14, that in primary complex, there is no significant depression of T cells in comparison with control, while other types of tuberculosis showed significant depression of T-cells. It is also evident from the table that maximum depression of T-cells occur in cases of tubercular meningitis.

Table 15

B-lymphocyte count by E-rosette formation in mantoux positive and mantoux negative BCG positive cases and in controls.

Groups	No.of cases	Range	B-lymphocyte percentage (Mean $\pm$ S.D.)
a) Control	10	15 - 28	20.1 $\pm$ 3.89
b) Mantoux positive	30	17 - 28	19.9 $\pm$ 2.7
c) Mantoux negative BCG positive	20	17 - 26	20.0 $\pm$ 3.33

$t_{a \times b} = 1.08$, $P > 0.05$

$t_{a \times c} = 0.08$, $P > 0.05$

$t_{b \times c} = 0.34$. $P > 0.05$

It is evident from table No. 15 that. in control group of cases, B cell percentage is 20.1 $\pm$ 3.89, while in the cases of mantoux positive and mantoux negative BCG positive cases the B cell values are 19.9 $\pm$ 2.7 and 20.0 $\pm$ 3.33 respectively. There is no significant

difference of B cell count in the cases of mantoux positive and mantoux negative BCG positive cases of tuberculosis. There is also no significant difference in mantoux positive and mantoux negative BCG positive cases.

Table 16

B cell percentage in different types of tuberculosis.

Type of tuberculosis	No.of cases	B cell % (Mean ± S.D.)
a) Control	10	20.1 ± 3.89
b) Primary complex	10	19.0 ± 1.67
c) Progressive Primary complex	5	19.0 ± 2.35
d) Tubercular consolidation	5	20.6 ± 2.30
e) Tubercular meningitis	10	20.7 ± 3.30
f) Milliary tuberculosis	5	21.0 ± 2.0
g) Cervical lymphadenitis	15	20.1 ± 2.77
Total number of cases	60	

$t_{a,b} = 1.38,\ P > 0.05$

$t_{a,c} = 1.76,\ P > 0.05$

$t_{a,d} = 0.42,\ P > 0.05$

$t_{a,e} = 0.49,\ P > 0.05$

$t_{a,f} = 0.80,\ P > 0.05$

$t_{a,g} = 0\ \ P > 0.05$

$t_{b,c} = 0\ \ P > 0.05$

$t_{b,d} = 0.58,\ P > 0.05$

$t_{,be} = 1.45,\ P > 0.05$

$t_{b,f} = 2.06,\ P > 0.05$

$t_{b,g} = 1.12,\ P > 0.05$

$t_{c,d} = 1.09,\ P > 0.05$

$t_{c,e} = 1.06,\ P > 0.05$

$t_{c,f} = 1.45,\ P > 0.05$

$t_{c,g} = 1.52,\ P > 0.05$

$t_{d,e} = 0.06,\ P > 0.05$

$t_{d,f} = 0.29,\ P > 0.05$

$t_{d,g} = 0.36,\ P > 0.05$

$t_{e,f} = 0.19,\ P > 0.05$

$t_{e,g} = 0.49,\ P > 0.05$

$t_{f,g} = 0.65,\ P > 0.05$

It is evident from table No. 16 that there is no significant difference of B cell count in between the cases of control and cases of different types of tuberculosis. There is also no significant difference of B cell count in between different forms of tuberculosis.

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DISCUSSION

DISCUSSION

The present study was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi, over a period of one year from September 1989 to August 1990. Three hundred suspected cases of tuberculosis were selected for present study and the diagnosis was confirmed by the preliminary investigation of Mantoux and B.C.G. diagnostic test. Accordingly, only those cases which were mantoux positive (81 cases) and mantoux negative B.C.G. positive (70 cases) were selected for further investigation. Further investigation done on these two groups of cases (151 cases), revealed that our case material comprised, 30 cases of primary complex, 21 cases of progressive primary complex, 22 cases of tubercular consolidation, 32 cases of tubercular meningitis, 12 cases of milliary tuberculosis and 24 cases of cervical lymphadenitis. However, immunological profile in these cases of childhood tuberculosis could only be conducted in 50 cases (10 cases of primary complex, progressive primary complex 5, tuberculosis consolidation 5, tuberculosis meningitis 10, milliary tuberculosis 5, cervical lymphadenitis 15) and 10 normal healthy immunologically competent children acted as control for the present study.

In the light of observation depicted from table 1 to table No. 16, the following references have been drawn and discussed.

The presenting features :

It is evident from table No. 1 that, out of the 300 suspected cases of tuberculosis selected for present study, superficial lymphadenitis was found to be present as presenting feature in most of the cases (80.66%). Thereafter, non-specific symptoms predominated (44.66%), which have been detailed earlier. Superficial lymphadenitis was most common presenting feature has also been observed by various other workers in the field (Lincon and Sewell, 1963; Bhakoo and Gupta, 1969, and Hutchinson, 1975).

Similarly, various other workers (Ramchandran and Purnayyan, 1966; Bhakoo and Gupta, 1969; Manchanda, 1969; Jain, 1978) too, like us have reported higher incidences of non-specific symptoms as observed by us. The higher incidence of superficial lymphadenopathy in childhood tuberculosis has been ascribed to the presence of rich lymphatic supply in neck region and also due to haematogenous spread which usually takes place in primary tuberculosis.

<u>Mantoux test</u> :

Since definitive diagnosis of tuberculosis can only be made by acid fast bacilli in the appropriate material which though not impossible, but extremely difficult in childhood tuberculosis (I.C.M.R., 1954; Rajnarain, 1963; Rajnarain et al, 1968; Unpublished data, tuberculosis prevention trials in Chingleput district, 1970), a positive mantoux test is still considered to be one of the most important screening test for early detection of disease. Accordingly, Mantoux test was performed in 300 suspected cases of tuberculosis and it was observed (Table No. 2) that 81 cases (27%) were mantoux positive, 80 cases (26.67%) were doubtful while 139 cases (46.33%) showed negative reaction. It is a well known fact, detailed in literature, that mantoux reactivity is governed by various factors of which the most important is malnutrition as has been observed by various workers (Udani et al, 1971; Mittal et al, 1977; Chandra et al, 1977; Seth et al, 1985). Udani et al (1971) studied mantoux positivity in various grade of nutritional status and found that in established cases of various types of tuberculosis in poorly nourished children, tuberculin test failed in 50% of cases and in suspected cases, 37.5% were non-reactor, whereas non-tuberculous cases 100% were non-reactor. Mittal et al (1977) also showed that mantoux test does not appear to be a reliable diagnostic index of

tuberculosis in 1TU strength as in his study, there was very low incidence of positive reactors in grade III and IV malnutrition (7.53% and 6.77%). In view of the high incidence of mantoux negativity even in established cases of tuberculosis, the importance of B.C.G. vaccination as a diagnostic test has come into vogue.

Thus B.C.G. vaccination was performed in both doubtful and negative mantoux reactors and it was observed (Table 3 and 4) that, of the 139 cases showing mantoux negativity, 70 cases (50.36%) showed accelerated response, which was considered as B.C.G. positive response, whereas BCG positive test was observed in 53 of the 80 cases of mantoux doubtful reaction (66.25%). Thus, on the basis of both mantoux and B.C.G. test done in the 300 suspected cases of tuberculosis, it was seen that only 204 cases were confirmed to have tuberculosis (mantoux positive 81 cases, mantoux negative BCG positive 70 cases and doubtful reactor BCG positive 53 cases). On further analysis of value of mantoux test as a diagnostic aid for tuberculosis as was observed by us, it is clearly evident that of the 204 cases of tuberculosis, only 81 cases had mantoux positivity (39.71%), while rest 60.29% cases showed mantoux negative reaction in spite of child being a case of tuberculosis. Similar observations of the value of mantoux test in diagnosis of tuberculosis has been detailed by various authors and results of their study are more or less in conformity to that of us.

Bhakoo et al (1969) recorded mantoux negativity of about 60 - 70%. Ramachandran et al (1976) and Buckly et al (1972) about 60%; Chandra et al (1977), however, showed a non-reactivity about 56%. An overall view of the mantoux test clearly signifies that mantoux test can not be relied as a diagnostic aid in all forms of tuberculosis. The slight difference in mantoux non-reactivity as observed by many workers may be attributed to difference of techniques, stroage and climatic condition.

From the above observations, regarding B.C.G. test as a diagnostic aid, viz. 50.36% response even in mantoux negative cases and 66.25% positive response in mantoux doubtful reaction, it is clearly evident that BCG test is more reliable as well as sensitive test for the diagnosis of childhood tuberculosis. While the mantoux test is governed by various factors mainly malnutrition and immuno-deficiency, BCG vaccination which is stronger antigenically is not governed by these factors and hence is considered to be more superior in the diagnosis of tuberculosis than mantoux test. The superiority of BCG test in the diagnosis of tuberculosis has also been confirmed by various other workers in the field (Udani et al, 1971; Desai et al, 1972; Lotte et al, 1973; Chaudari et al, 1974; Chandra et al, 1977).

Mantoux reaction in Malnourished children :

Since with our observation and that of other workers it has been clear that the mantoux reactivity is governed by malnutrition, we went a step further and tried to establish a relation of the mantoux reactivity in different grades of malnutrition. It was observed from Table No. 5 that out of 125 cases of malnutrition, 59 (47.2%) were mantoux positive. Out of 36 cases having grade I malnutrition, mantoux was positive in 26 cases (72.22%). Out of 42 cases Grade II malnutrition, mantoux was positive in 22 cases (52.38%). In grade III and grade IV malnutrition, mantoux positivity was in 8 and 3 cases out of 32 and 12 cases respectively. It is evident from our study that mantoux positivity was maximum in grade I malnutrition and subsequently decrease as severity of malnutrition increase. Udani et al (1971) studied tuberculosis reactivity in various grades of malnutrition and found that 37.5% were non-reactor in suspected cases of tuberculosis. Mittal (1977) observed maximum positivity in grade II type of malnutrition and lower percentage in grade III and grade IV malnutrition. This low percentage of tuberculosis positivity is firstly because of using 1 TU strength (Mittal et al, 1977). Secondly, malnutrition causes immuno-deficiency so as grade of malnutrition increases the immuno-deficiency increases and this causes decrease in positivity of mantoux test.

Since malnutrition per se has a direct impact on the immune status of a child, it was extremely important for us to assess the nutritional status of our cases of childhood tuberculosis so that the impact of both nutritional status as well as tuberculosis could be delineated in each case. Accordingly, we tried to assess the nutritional status in whole the 131 cases of childhood tuberculosis in our study. It is evident from Table No. 6 that there was a correlation with severity of tuberculosis with degree of malnutrition, while in primary tuberculosis 50% cases had normal, and grade I and grade II malnutrition each. In the disseminated form of tuberculosis grade III and grade IV malnutrition predominated (Tubercular consolidation 22.73% and 13.63%, tubercular meningitis 16.7% each and milliary tuberculosis 16.7% each). Very few workers in literature tried to evaluate the effect of malnutrition and tuberculosis vis-a-vis the immunological profile. Seth et al (1985) however like us, have reported normal nutrition in 48%, and grade I and II malnutrition in another 48% of cases. Seth and Singh (1987) in another study however reported a greater incidence of grade I and II malnutrition (50.5%) as well as grade III malnutrition in 10.5% of cases. Both these workers have like us also reported increase in severity of malnutrition as the severity of tuberculosis increases (33.3% grade III and grade IV both in milliary and tubercular meningitis, 36.4%

in tubercular consolidation, 17.6% in cervical lymphadenitis, 14.3% in progressive primary complex, 9% in primary complex).

Haematological Investigations :

Haematological investigation was carried into 81 cases of mantoux positive cases and 70 cases of mantoux negative BCG positive cases.

In the present study, normal values of total leucocyte count, absolute lymphocyte count, erythrocyte sedimentation rate and haemoglobin have been taken as haemoglobin more than 10 gm%. Total leucocyte count between 6,000 - 10,000 cells/cmm, absolute lymphocyte count between 1500 - 3500 cells/cmm, ESR values of 0 - 20 mm by Wintrobe method. Leucocytosis in 106 (70.19%) cases, absolute lymphocytosis in 91 (60.26%) cases, haemoglobin less than 10 gm% in 101 (66.88%) cases and raised ESR in 108 (71.52%) cases were found in out of 151 cases. Our figures of haemoglobin are in confirmation with findings of Mukerjee (1966), Udani (1968) and Srivastava (1980). Wattal (1979) reported haemoglobin less than 10 gm% in 95.33% of cases of tuberculosis. Percentage of cases showing leucocytosis is higher than reported by Udani (1968) and Srivastava (1980). Wattal reported leucocytosis and relative lymphocytosis in 93% of cases. Our finding of raised erythrocyte sedimentation rate is less (71.52%) than reported by Udani (1968) and Srivastava (1980).

On comparison of mantoux negative BCG positive and mantoux positive, Hb less than 10 gm% was present in 51 cases (72.85%) and 50 cases (61.72%) while more than 10 gm% was present in 19 (27.15%) and 31 (38.28%) of cases respectively. Our this finding is in confirmation with Srivastava (1980). High incidence of reduced Hb in mantoux negative cases of tuberculosis can be explained by this fact that maximum number of mantoux negative BCG positive cases were suffering from severe grade of malnutrition. There was no remarkable difference in leucocyte count and in lymphocyte count in between mantoux negative BCG positive cases and mantoux positive cases. Percentage of raised ESR was more in mantoux positive cases. Many workers have shown that there is not more significance of haematological investigations in the diagnosis of tuberculosis. The lowered haemoglobin percentage suggests the association of a chronic infection with or without malnutrition with iron deficiency. Leucocytosis e.g. lymphocytosis also suggests chronic nature of the disease. Raised ESR is not a direct diagnostic value but it confirms the presence of active disease and serial estimation are valuable in assessing the prognosis of cases during the treatment (Chutchison, 1975).

Cellular immunity in various study groups :

The cellular immunity was assessed by the T lymphocyte count (E-rosette formation) in our three broad

group of cases. It is evident from table No. 11 that in the 10 control group of cases, E-rosette count was 60.8 ± 3.8, with a range of 59 to 70, whereas in 30 cases of mantoux positive and 20 cases of mantoux negative but BCG positive cases, the percentage of T lymphocyte count was 57.13 ± 6.43, and 40.25 ± 5.47% with a range of 45 - 68 and 30 to 54 respectively.

Thus our observation revealed that there was maximum depression of cell mediated immunity in mantoux negative BCG positive cases, when compared to mantoux positive and control group. On statistical analysis, it was seen that only mantoux negative BCG positive cases had statistically significant difference from the control values ($P < 0.001$), whereas no statistical difference was observed between mantoux positive and the control group ($P > 0.05$). A comparison of the mantoux positive and mantoux negative BCG positive cases, in the T cell count, however, showed a significant difference ($P < 0.001$). The greater depression of cellular immunity as measured by T lymphocyte count in mantoux negative BCG positive cases observed by us, is due to the fact that of the 20 cases in this group, 15 cases were of disseminated form of tuberculosis and most of them as detailed earlier were having severe grade of malnutrition. Similar observation of depression of E-rosette in cases of tuberculosis has been found by various workers (Bhatnagar et al, 1977; Tanphachitra, 1979; Seth et al, 1981; Seth et al, 1985; Mathur et al, 1989).

The decrease in T cell count in cases of tuberculosis has been ascribed by these worker due to depression of cell mediated immunity in cases of tuberculosis as well as by the malnutrition which is many a times associated with tuberculosis (Simpson et al, 1953; Boughton et al, 1963; Reddy et al, 1977; Seth et al, 1985).

Seth et al (1985) found more depression in T cell count in severe type of tuberculosis in which the severe grade of malnutrition was present. Mathur et al (1989) studied T cell count in mantoux positive and mantoux negative BCG positive cases and they found that T cell count was significantly decrease in mantoux negative BCG positive cases ($P < 0.05$).

Further, we also tried to assess the T lymphocyte count in the various forms of childhood tuberculosis to ascertain whether the type and survey of tuberculosis has any effect on the immunological profile of the disease, which has been shown in Table No. 14. It was evident from table that maximum depression of T cell count was seen in cases of tubercular meningitis (39.9 ± 5.09), followed by cases of milliary tuberculosis and tubercular consolidation in which T cell count was 46.4 ± 7.55, 49.6 ± 11.07 respectively. On statistical analysis, it was evident that besides primary complex, which did not show any statistically significant decrease than the control group of cases ($P > 0.05$). All the other forms of tuberculosis had a significant difference from the control though the significance was

maximum in cases of tubercular meningitis and milliary tuberculosis.

Thus, an important significant finding of our study is that disseminated form of tuberculosis had more depression of the cellular immunity has also been observed by umpteen workers in past (Bhatnagar et al, 1977; Chernushenko et al, 1982; Seth et al, 1985). Mathur et al (1989) in their study did not try to observe the correlation of the cellular immunity in the different forms of tuberculosis. Chernushenko et al (1982) and Seth et al (1987) were also of the opinion like us that there was no significant difference in B cell count in various types of tuberculosis when compared to normal health control. However, Seth et al in another study done singularly in the cases of cervical lymphadenitis did show depression of B count in these cases, while Venkata Reddy et al (1985) found however unlike us significant increase in B cell percentage in cases of tuberculosis. Explanation of the decrease or increase of B cell sub-population in cases of tuberculosis has been forthcoming by these workers. Like the T cell count, we also try further EAC rosette count in the different forms of tuberculosis in this study. Unlike the T cell count, however there was no correlation between severity of disease and B cell counts. Similar observation is shown by Chernushenko (1982) and Seth et al (1987).

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SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi, over a period of one year. Three hundred children suspected of having various types of tuberculosis in the age group of less than 12 years, attending Paediatrics outdoor department, Well Baby Clinic and those admitted in Paediatrics ward were included in this study. The study was designed to know delayed hyper-sensitivity in the form of mantoux and B.C.G. test as well as to study the cellular immune response in the form of T and B cell. We also tried to see the association of malnutrition as well as effect of malnutrition on delayed hyper-sensitivity and cellular immune response in childhood tuberculosis.

The cases were subjected to detailed history taking, a thorough general and systemic examination and mantoux test.

On the basis of induration of Mantoux test, cases were divided into three groups -

1. Mantoux positive (induration more than 10 m.m.). These cases were investigated for tuberculosis and proved for tuberculosis.

2. Doubtful reactors (induration between 5-9 m.m.).

3. Negative mantoux test (less than 5 m.m.).

Doubtful reactors and mantoux negative BCG positive cases were also given B.C.G. vaccine and accordingly cases showing induration below 5 m.m. with B.C.G. vaccination were left as such assuming as non-infected cases, while accelerated responders (induration 5 m.m. or more) were investigated for tuberculosis and categorised into different forms of tuberculosis.

Further investigation such as haemoglobin, total leucocyte count, differential leucocyte count, erythrocyte count, X-ray chest and other specific investigation like CSF were done to establish the diagnosis of disease.

In this study, out of 300 suspected cases of tuberculosis, 204 cases proved as cases of tuberculosis (81 cases mantoux positive, 70 cases mantoux negative BCG positive and 53 cases of doubtful reactor but BCG positive).

It is clearly evident from our observations that mantoux positivity was found only in 39.71% suspected cases of tuberculosis, while more than half of the cases (60.29%) in spite of clinical suspicion of tuberculosis showed a negative response, causes of which were supposed to be malnutrition and immuno-deficiency.

The value of B.C.G. vaccination as a diagnostic test superior to mantoux test was highlighted by our observation that out of 139 cases which were mantoux negative, 70 cases (50.36%). Out of these, on B.C.G. vaccination showed an accelerated response which were designated as B.C.G. positive. Similarly, 66.25% B.C.G. response was also observed in mantoux doubtful cases. These two observations clearly indicate the superiority of BCG test over that of mantoux in the diagnosis of cases of childhood tuberculosis specially in cases of malnourishment and immuno-deficiency.

A correlation of the mantoux reactivity in different grades of malnutrition revealed that mantoux positivity was maximum in grade I malnutrition (72.22%), and minimum (20%) in grade IV malnutrition. This can be easily explained on the basis of cellular immune deficiency, which is prevalent in increasing grades of malnutrition.

The nutritional status of proved cases of tuberculosis in our study was assessed in each cases of childhood tuberculosis so as to ascertain the effect of malnutrition per se immune status. We observed that, there was a direct correlation between severity of tuberculosis to the degree of malnutrition. In the disseminated form of tuberculosis grade III and grade IV predominated (22.73% and 13.63% tubercular consolidation) tubercular meningitis (16.7% each) and milliary tuberculosis (16.7% each).

Cellular immunity :

The cellular immunity could only be assessed into 10 healthy control group of cases, 30 mantoux positive cases and 20 cases which were mantoux negative but BCG positive. Cellular immunity in our study was assessed by T lymphocyte count by E-rosette test. It was evident from our observation that in 10 control group of cases, E-rosette count was 60.8 ± 3.8 with range of 59 - 70. In 30 mantoux positive cases, T lymphocyte count was 57.13 ± 6.43 with a range of 45 - 68 whereas in 20 mantoux negative BCG positive cases, T lymphocyte count was 40.25 ± 5.47 with a range of 30 - 54. Our observations clearly revealed that mantoux negative BCG positive cases had maximum depression of cellular immunity as assessed by T lymphocyte count (then control and mantoux positive cases) because, out of the 20 cases in the group 15 cases were of disseminated form of tuberculosis and most of them were in grade III and grade IV malnutrition, both factors predisposing to cellular immune deficiency.

Like the T cell count, we tried to do EAC count (B cell) and we observed that there were no significant difference in B cell count in mantoux positive and mantoux negative BCG positive cases when compared to normal healthy control. We also further tried to assess EAC rosette count in different types of tuberculosis. Unlike the T cell count, however, there was no correlation between the severity of disease and B cell count.

In a nutshell, our study has the following message to convey -

1. BCG vaccine as a diagnostic test was found to be more superior than mantoux test specially in the cases of malnutrition and immuno-deficiency.

2. Cellular immunity as assessed by T lymphocyte count was depressed in cases of childhood tuberculosis than normal healthy children. A significant finding was that the severity of depression of cellular immune response was in direct correlation with the severity of tuberculosis.

3. B cell count, which is an index of humoral response, however, was not found to be affected in cases of tuberculosis.

The mechanism of depression of cell mediated immune response which has been observed in our study is due to two important factors -

(a) Association of malnutrition (in cases of tuberculosis) which is a well documented factor in causation of depression of cell mediated immune responses.

(b) The tubercular infection per se is also considered to be an important factor in depression of cell mediated immunity. There is unending literature to explain the pathogenesis of depression of cell mediated immune responses in tuberculosis. Seth et al (1987) have

documented that the virulence of tubercular bacilli in the causation of tissue damage is due to three virulent components in mycobacteria tuberculosis, viz. sulphatides, C-mycoside and cord factors. All these factors are seen to have an adverse effect either on the afferent or the efferent limbs of cellular immunity. It is seen that these factors inhibit the activation of new macrophages (which is the first line of defence) initially. Seth et al (1987) is of the opinion that the cell mediated immunity in tuberculosis is due to activation of new macrophages by lymphokines liberated by antigen specific helper T cells and these new macrophages act over mycobacterium. It is seen that if the macrophages are not activated, the tubercular bacilli continue to divide within the macrophages and more proteoliolytic enzymes are liberated into extra cellular space resulting in further destruction of host tissue. Further, these workers have hypothesised that the depression of T cells may be due to either exposure to large levels of antigen, or clonal deletion, blockage of specific receptor sites or due to formation of antigen antibody complexes.

However, in addition to the above findings, a detailed study of the sub-population of T cells, assay of different lymphokines in vitro is needed to unravel the mechanism of tuberculo-immunity in children.

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BIBLIOGRAPHY

BIBLIOGRAPHY

1. Achar, S.T. and Vishwanathan, J. : 'Abdominal tuberculosis'. Text book of Tuberculosis, Ist. Ed., P. 361, 1972.

2. Adams, D. : Molecules membranes and macrophage activation. Immunol. Today, 3, 228, 1982.

3. Archer, O.K., Sutherland, D.E.R. and Good, R.A. (1964) : Quoted from Harrison, C.V. and Weinbery, K., Recent Advances in Pathology, Churchill Livingstone, Lond-1973.

4. Aronson, J.D. : Amer. Rev. Tuberc., 63 : 121, 1951.

5. Barua, B.N.M. : Proceedings of XIX Tuberculosis and Chest disease workers Conf., Delhi, 1964.

6. Barucha, B.S. and Deshpande, D.M. : Tuberculous meningitis in children. Indian Pediatr., 6 : 282, 1969.

7. Berry, J.N. and Sohdar, R.J. : Diagnosis and treatment of tuberculosis meningitis. J.of Asso. Phy. India, 5 : 191, 1957.

8. Bhakoo, O.N. and Gupta, S.P. : "Tuberculosis in Children". Ind. J. Paed., 36 : 65, 1969.

9. Bhargava, S.K., Saha, M.M., Agarwal, K.M. and Rao, V. : "Some observations on the pattern of Pulm. Tub. in Children" Ind. Paed., 4 : 262, 1967.

10. Bhatnagar, R., Malviya, A.N., Narayan, S., Rajgopalan, P. Kumar, R. and Bharadwaj, O.P. : Am. Resp. Dis., 115 : 201-212, 1977.

11. Bogen, E. : "The myobacteria of India". Ind. J. Chest Dis., 2 : 143, 1960.

12. Boughton, B. and Spector, W.G. (1963) : Quoted from the lymphocyte, Lloyd-Luke (Medical Books) Ltd., Lond., 1972.

13. British Tuberculosis Association (1959) : "Grading of mantoux reaction". "Quoted by Miller, F.J.W., Seal, R.M.E. and Taylor, M.D., Tuberculosis in children". P. 83, 1963, J & A Churchill Ltd.

14. Bruce, R.A. : "Observations of the measurement of skin hypersensitivity of tuberculin." Tubercle (Lond.), 42 : 199, 1961.

15. Bruyn, (1945) : Quoted in Lloyd Luke (Medical Books).

16. Buckley, C.E. and Vilseck, J.R.,: Anergey in active Tuberculin (Extract). Society of Immuno-clinical Research, 20 : 79, 1972.

17. Bullock, W.E. (1974) : Immunodeficiency in Leprosy and other infections. Progress in immunology, II Vol. 5, P. 193, Ed. Brent. L. and Holborow.

18. Calmette, A. and Guerin, C. (1902, 1921, 1924, 1933) : Quoted by Harper, P.A. : "Preventive paediatrics". P. 410, 1962. Appleton Century Crafts, New York.

19. Calmette, A. and Guerin, C. : Compt. Ren. Acad. 147 : 1956, 1908. Quoted by Friedman, S. and Silverman, I.: B.C.G. Vaccination as a new Diagnostic Test for T.B.". Pediatr., 9 : 280, 1952.

20. Calwell, H.G. : Temporary suppression of Tuberculin sensitivity Tubercle (Lond.), 38 : 287, 1957.

21. Chan, S.H., Lee, S.K. and Sions, M.J. : Levamisole augmentation of lymphocyte hyporesponsiveness to Phytohaemagglutinin in patients with Pulm. Tub. Proc. Soc. Exp. Biol. Med., 151 : 716, 1976.

22. Chandra, R.K. : "Immuno-competence in under-nutrition". J. Paed., 81 : 1194, 1972.

23. Chandra, K., Praharaj and Choudhury, U. : Evaluation of mantoux and B.C.G. test in the diagnosis of childhood tuberculosis. I.P., 14 : 99-102, 1977.

24. Chaudhary, V.P., Singh, M.M. and Verma, I.C. : "B.C.G. and mantoux intradermal test in diagnosis of Tuberculosis". Ind. Paed., 11 : 535, 1974.

25. Chernushenko, E.F., Mamolat, A.S., KoGosoba, L.S. and Petrushenbo, A.T. : State of immunological reactivity in tuberculous patients. Indian J. Chest Dis. Allied Sci., 24 : 158-163, 1982.

26. Citron, K.M. and Scadding, J.G. : 'The effect of cortisone upon the reaction of the skin tuberculin in the T.B. and in sarcoidosis'. Quart. J. Med., 26 : 277, 1965.

27. Cooper, M.D., Peterson, R.D.A. and South, M.A. : J. Exp. Med., 123 : 75, 1966.

28. Crowle, A.J. and May, M.H. : Preliminary demonstration of human tuberculo-immunity in vitro. Infec. and Immunity, 31 : 453, 1981.

29. Crowle, A.J., Douvas, G.S. and May, M.H. : The cellular and molecular nature of human tuberculo-immunity. Bull. IUAT., 58 : 72, 1983.

30. Dannenberg, A.M. : Pathogenesis of pulmonary tuberculosis. Amer. Rev. Respir. Dis., 125 : 25, 1982.

31. Davis, A.J.S. (1969) : Quoted from Harrison, C.V. and Weanbren, K. 'Recent advances in Pathology', Churchill Livingston, 1975.

32. Davis, D.A.L., Albius, B.J., Boyse, E.A., Old, L.J. and Stocbert, E. : Immunol., 16 : 609, 1969.

33. Desai, A.B., Bami, G. and Ahya, P.N. : 'Diagnostic value of B.C.G. in Tuberculosis'. Ind. Paed., 9 : 767, 1972.

34. Diagnostic Standard (1961) of the National Tuberculosis Association, America. "Interpretation of Tuberculin Test" Quoted by Nelson, W.E. Text book of Paediatrics, 9th Ed., 1969. P. 604, W.B. Saunders Co., London.

35. Diagnostic Standard and Classification of Tuberculosis, New York, American Lung Association (1969) : "Interpretation of Mantoux Test". Quoted by Vaughan, V.C., Koy, M.C.W. and Nelson, R.J. 'Text book of Paediatrics', 10th Ed., 1975, P. 634, W.B. Saunders Co., Ltd., London.

36. Dick, W.P. : "The value of Tuberculin Jelly Test". Brit. Med. J., 2 : 141, 1950.

37. Dingley, H.B. : "Tuberculosis in India". Ind. Paed., 13 : 879, 1976.

38. Dixit, K.P. and Singh, S. : "B.C.G. Test for diagnosis of Childhood Tuberculosis". Ind. Paed., 13 : 687, 1976.

39. Douglas (1960) : Quoted by Vishwanathan, R. Pulm. Tub. Ass. Pub. House, 1966.

40. Elvis, M.W. : The lymphocyte Lloyd-Luke (Medical Books) Ltd., London, 1972.

41. Everett, N.B. and Tyler, R.W. (1967) : "Quoted for recent advances in Pathology", Churchill Livingston, London, 1975.

42. Ford, W.L. (1969) : "Recent advances in Pathology". Churchill Livingston, London, 1975.

43. Frappier, A. and Guy, R. : "New and practical B.C.G. skin test (B.C.G. scarification) test for detection of total tuberculous allergy". Canad. J. Pub. Health, 41 : 72, 1950.

44. Friedman, E. and Silverman, I. : Use of BCG vaccine as a New Diagnostic test for Tuberculosis". Paediatr., 9 : 280, 1952.

45. Froland, S., Natvig, J.B. and Bergal, P. : Nature (New Biol.), 234 : 251, 1971.

46. Godal, T., Myklestad, B., Sammuel, D.R. and Myrvang, B. : Characterization of the cellular immune defect in Lepromatous leprosy : A specific lack of circulating Mycobacterium leprae reactive lymphocytes. Clin. Exp. Immun., 9 : 821-833, 1971.

47. Godal, T., Lofgren, M. and Negassi, K. : Immune response to M. leprae of healthy leprosy contacts. Internat. J. Leprosy, 40 : 243-250, 1972.

48. Gowans, J.L., Knight, E.J. : The route of re-circulation in the rat. Proc. Roy. Soc. (London), Series B, 159 : 257, 1964.

49. Gowans, J.L. : Lymphocytes. The harvey lectures, 64 : (1968-1969), 87, 1970.

50. Grange, J.M. : Some aspects of immunology relevant to paediatric tuberculosis. Pediat. Clin. of India, 18 : 50, 1983.

51. Griffiths, A.H. : 'B.C.G. vaccination by multiple puncture method'. Lancet 1 : 1170, 1959.

52. Hall, J.W. and Furth, J. : Arch. Path., 24 : 46, 1938.

53. Human, L.V. and Wolman, S. : "Tuberculin : Diagnosis and Treatment". P. 380, 1912. Appleton, century. New York. Quoted by Wills, H.S. and Cummings, M.M. "Diagnostic and experimental methods in T.B.". P. 184, 1952. 2nd Ed. Charles, C. Thomas Publisher, U.S.A.

54. Hutchinson, J.H. : "Tuberculosis". Practical Paediatrics Problems, 4th Ed. P. 160-163, 1975, Llyod Luke Ltd., London.

55. Harland, P.S.E.G. : Tuberculin reaction in malnourished children. Lancet 2 : 719, 1965.

56. Heaf, F.R.C. and Russy, N.L. : Recent Advances in Resp. Tuberc. J. and of Churchill Ltd. London, Chapter IV, P. 84, 1959.

57. Heaf, F. : "The Multiple puncture tuberculin test". Lancet, 2 : 151, 1951.

58. Hsu, H.S. : "Cellular basis of cortison induced host susceptibility to Tuberculosis". Amer. Rev. Resp. Dis., 100 : 677, 1969.

59. Indian Council of Medical Research (1959) : "Tuberculosis in India - A sample survey 1955-58". I.C.M.R., New Delhi.

60. Jain, C.S. : Comparative study of BCG and mantoux intra-dermal tests in diagnosis of tuberculosis in infancy and childhood. Thesis for M.D.(Paed.), Agra University, 1978.

61. Jaiswal, S. and Bhandari, N.A. : "Evaluation of diagnostic value of B.C.G. Test in childhood tuberculosis. Ind. Paed., 13 : 689, 1976.

62. Jakubusek, M.P. and Junica, G. : Bu. Jr. Resp. Dis., 6112 : 67-70, 1980.

63. James, A., Neidhard, M.D., Norma Christakis, M.D., Earl, N., Metz, M.D., Stanley, P., Bakerzak, M.D. and Albert F. Lobuglio, M.D. : Skin test conversion following transfer factors. The Journal of Allergy and Clinical Immunology, 61 : 115-118, 1977.

64. Jondal, M. et al : Human lymphocytes sub-population classification according to surface markers and/or functional characteristics. Transplant Review, 16 : 163, 1973.

65. Jondal, M., Holm, G. and Wizgell : Surface markers of human 'T' and 'B' lymphocytes. J. Exper. Med., 136 : 207, 1972.

66. Kanai, K. and Kondo, E. : Sub-cellular and inter-cellular aspect of tuberculous infections in reference to protection and sensitization. Japanese J. of Med. Scien. and Biology, 25 : 133-161, 1972.

67. Lay, W.H., Mendes, N.F., Bianco, C. and Nussenzweig, V. : Nature, 230 : 531, 1971.

68. Lincon, E.M., Sordillo, V.R. and Davies, P.A. : A review of 167 untreated and 41 treated patients with special reference to early diagnosis. J. Paed., 57 : 807, 1960.

69. Lincon, E.M. : "TBM in children with special reference to serious meningitis". Amer. Rev. Tubercle, 56 : 7, 1974.

70. Lincon, E.M. and Swell, E.M. : Tuberculosis in children P. 10-11, 165-166, 1969-170, 1963, McGraw Hill Co. Inc. New York.

71. Long, E.R., Seibert, F.B. and Aronson, J.D. : A standardised tuberculin PPD for uniformity in diagnosis and epidemiology. Tubercle (Lond.), 16, 304, 1935. Quoted by Miller, F.J.W., Seal, R.M.E. and Taylor, M.D. "Tuberculosis in children". P. 790. J. and A. Churchill Ltd., London, 1963.

72. Lorbes, J. : "B.C.G. Tuberculin". Arch. Dis. Child., 32 : 441, 1957.

73. Lothe, P.S., Gupta, S.P. and Agarwal, B.P. : "Evaluation of B.C.G. as a diagnostic test in T.B.". Ind. Paed., 10 : 419, 1973.

74. Mackaness, G.B. : Resistance to intra-cellular infection. J. of Inf. Dis., 123 : 439, 1971.

75. Mackaness, G.B. : The immunology of anti-tuberculous immunity. Amer. Rev. Resp. Dis., 97 : 337, 1968.

76. Mathur, P.P., Dayal, R., Prasad, R., Agarwal, A., Ethence, Dayal and Pandey : T lymphocyte in children with tuberculosis. Indian Paediatrics, 26 : 383-386, 1989.

77. Malviya et al : DNCB skin sensitisation and its relationship with PPD reactivity in pulmonary tuberculosis. Ind. J. Med. Res., 61 : 885, 1973.

78. Manchanda, S.S. : Tuberculosis in children below the age of 3 years. Ind. J. Child Health, 11 : 8, 1969.

79. Manchanda, S.S., Bawa, Y.S. and Bhatia, J.L. : Tuberculosis in children - A preliminary report of 215 cases. Ind. J. and Child Health, 7 : 938, 1958.

80. Manchanda, S.S. and Lal, H. : Tuberculous meningitis unsolved in India. Indian Pediatr., 3 : 167, 1966.

81. Mande, R. : Manual Practice de vaccination Parle B.C.G. Centre international de 1. Enference Paras (Quoted by Griep, W.A. and Bleiker, M.A. Tubercle (London), 38 : 259, 1957.

82. Mantoux, C. (1908) : Quoted by Wills, H.S. and Cummings, M.M. : "Diagnostic and experimental methods in Tuberculosis". P. 20, 1952, 2nd Ed., Charles C. Thomas, Publishers, U.S.A.

83. Master, J. : Ind. J. Child Hlth., 4 : 576, 1955 (Quoted by Calwell, H.G. Tubercle (London), 37 : 287, 19

84. Matsaniotis, N., Tsenghi, C., Economan, Mavro, C. and Metaxolve, S.C. : Skin hyper-sensitivity and in vitro lymphocyte reactivity to tuberculin in childhood. J. of Paed., 79 : 399, 1968.

85. Miller, F.J.W. : The prevention and treatment of tuberculosis in children. Practitioner (London), 204 : 11, 1970.

86. Miller, F.J.W., Seal, R.M.E. and Taylor, M.D. : Tuberculosis in children. P. 78 : 396, 1963. J. and A. Churchill Ltd. London.

87. Mittal, S.L., Bhandari, N.R. : A study of tuberculin test in paediatric practice. Indian Paediatrics, 14 : 207-210, 1977.

88. Mittal, V.N. and Gupta, M.C. : "Tuberculosis meningitis". Ind. J. Med. Sci., 18 : 854, 1967.

89. Mukerjee, D.P. : Evaluation of combined corticosteroids and anti-tuberculous drugs in TBM. Thesis for M.D. (Paed.), Agra University, 1966.

90. Murray, R.G. (1947) : Quoted from the 'Lymphocyte'. Llyed Luke (Medical Books) Ltd., London, 1972.

91. Narain, R., Gaser, A., Jambunathan, M.V. and Subramaniam, M.: Tuberculosis prevalence survey in Tumkur district. Indian Jr. Tuberculosis, 10 : 85, 1963.

92. Nowell, P.C. et al : Phytohaemagglutinin an initiator of mitosis in cultures of normal human leucocytes. Cancer Res., 20 : 462, 466, 1960.

93. Nowell, P.C. : Blood, 26 : 798, 1965.

94. Nussenzweig, V., Bianco, C. and Durper, P (1971) : Membrane receptors for antigen-antibody complement complexes on lymphocytes. Cell interactions and receptor antibodies in human responses. In proceeding of the Third Sigrid Juselius Symposium. Academic Press Inc. New York, 1975.

95. Oort, J. and Turk, J.L. (1965) : Quoted from Harrison, C.V. and Weinbrn, K. : Recent advances in Pathology. Churchill Livingston, London, 1975.

96. Otteson, J. : On the age of human white cells in peripheral blood. Acta Physiol. Scand., 32 : 75, 1954.

97. Otteson, J. : (1954) : Quoted from Harrison, C.V. and Weinbren, K. : Recent Advances in Pathology. Churchill Livingston, London, 1975.

98. Palmer, C.E. and Edwards, L.B. : Jr. of the Amat. Med. Asso., 205 : 167, 1968.

99. Palmer, C.E. and Long, M.W. : Amer. Rev. of Resp. Dis., 94 : 53, 1966.

100. Papamichail, M., Halborow, E.J., Keith, H.I. and Currey, H.L.F. : Lancet 2 : 64, 1971.

101. Praharaj, C.K. and Chaudhary, U. : Evaluation of mantoux and B.C.G. test in diagnosis of childhood tuberculosis. Ind. Paed., 14 : 99, 1977.

102. Raj Narain et al : Bulletin of the World Health Organisation, 34 : 623, 1966.

103. Raj Narain, : "Interpretation of the report on tuberculin test. Tubercle (London), 49 : 85, 1968.

104. Raj Narain, : Tuberculosis prevalence survey in Tumkur District. Ind. J. Tuberc., 10 : 85, 1968.

105. Raj Narain et al : Ind. Jr. of Med. Res., 62 : 1896, 1974.

106. Raju, V.B., Narmada, R., Shanmugasundram, R. and Sambandam, : Crude mortality and tuberculosis morbidity among infected and un-infected group. Indian 8 : 11, 1971.

107. Ramachandran, R.S. and Purnayyan, S. : Tuberculosis in children. Ind. Paed., 3 : 218, 1966.

108. Ramachandran, R.S., Ramnathan, K. and Indra, G. : Tubercular Meningitis. Ind. Ped., 37 : 85, 1970.

109. Rich, A.R, (1946) : Pathogenesis of tuberculosis. Charles, Thomas Illinois, Chapter V.

110. Rich, A.R. : The pathogenesis of tuberculosis, 2nd Ed., Springfield, Thomas, P. 468, 1950.

111. Robert, H.H. : The tuberculin test. Nelson Text Book of Paediatrics, 10th Ed., P. 633, WBSaunders Co. London, 1975.

112. Roitt, Ivan. : Essential immunology, Blac-well scientific publications, Oxford London, Edinborough, Malbourne, 1974.

113. Rook, G.A.W. : Immunity and hyper-sensitivity. Practitioner, 227 : 4, 1983.

114. Salmen, H. and Angel, I.F. : Corticotropin induced changes in the tuberculin skin test. A controlled study in advanced T.B. Amer. Rev. Resp. Dis., 83 : 253, 1961.

115. Seibert, F.B. : Isolation and Properties of PPD. Amer. Rev. Tuber. (30 : 713, 1934). Quoted by Millen F.J.W., Seal, R.M.E. and Taylor, M.D. 'Tuberculosis in children', P. 79, 1963.

116. Sen Gupta, S.K. and Kapoor, P.N. : Pattern of morbidity and mortality in India with special reference to communicable diseases, Central Bureau of Health Intelligence, Ministry of Health and Family Planning, New Delhi, 1971.

117. Seth, V., Malaviya, A.N., Sahai, V., Arora, N. and Sundaram, K.R. : Cell mediated immune response in childhood tuberculosis. Ind. J. Med. Res., 73 : 68-73, 1981.

118. Seth, V., Rohatagi, M., Bhuyan, U.N., Sundaram, K.R. and Neera Nath : Tuberculous cervical lymphadenitis in children as a relatively immune competent state. Ind. J. Med. Res., 81 : 364-371, 1985.

119. Seth, V., Nath, N. and Singh, U. : Immune spectrum of childhood tuberculosis. Indian J. Tuberc., 32 : 29-39, 1985.

120. Seth, V. and Singh, U. : Immunopathogenesis in tuberculosis Part II : Humoral mechanism of resistance. Ind. J. Pediatr., 54 : 830-840 .311-319 1987.

121. Shah, P.M. and Udani, P.M. : Medical examination of rural school children in Palghar Taluk. Ind. Paed., 5 : 345, 1968.

122. Shevach, E.M., Heberman, R., Frank, M.M. and Green, I. : Receptor for Complement and immunoglobulin on human leukaemia cells and human lymphoblastoid cell lines. J. Clin. Invest., 51 : 1933, 1972.

123. Sikand, B.K. and Pamra, S.P. (1964) : Ind. J.J. Tuberc. XII, 3.

124. Singh, K.P. and Gulati, P.V. : Status of B.C.G. vaccination in children. Ind. Paed., 13 : 683, 1976.

125. Simpson, M., Buemann, J., Gamme Toff, A., Jenson, F. and Jorgensen, K. (1963) : Quoted from the lymphocyte Lloyed-Luke (Medical Books) Ltd., London, 1972.

126. Smith, H.V. and Vollums, R.L. : The diagnosis of T.B.M. Brit. Med. Bull., 10 : 140, 1954.

127. Smith, R.F. Neg. : J. Med., 278 : 1207, 1968.

128. Smith, R.W., Jerry, W.F., Bull, D.M. and Bell, K.W.J. : Immunol., 100 : 884, 1973.

129. Srivastava, V.K. : B.C.G. vaccination and tuberculin status in infancy and early childhood. A thesis for Doctor of Medicine (Paed.), Agra University, 1980.

130. Starr, S. and Berkorich, S. : Effects of measles, gamma-globulin modified measles and vaccine measles on the tuberculin test. N. Eng. J. Med., 270 : 386, 1964.

131. Stegen, C., Jones, K., and Kaplan, P. : Criteria for guidance in diagnosis of T.B. Pediatrics, 43 : 260, 1969.

132. Sunakorn and Azuma (1966) : Mimeographed document, WHO/ Tuberculosis. Tech. Information, 66-67.

133. Thomas, D.B. : Lancet, 1 : 399, 1972.

134. Thomson, R.B. : Disorders of the blood - A text book of Clinical Haematology, Churchill Livingstone, London, P. 490, 1977.

135. Tripathi, S.P. : Chemical constituent of M. tuberculosis. Text book of tuberculosis, 2nd Ed., P. 90, Kotari Book Depot, Bombay, 1972.

136. Trudeau, E.L.L. : Experimental study of Preventive inoculation in Tuberculosis. M. Rec. 38 : 565, 1890. Quoted by Willis, H.S. and Aimmings, M.M. Diagnostic and experimental methods in tuberculosis. P. 184, 1952 2nd Ed. Charles C. Thomas Publishers, U.S.A.

137. Turk, J.L. and Bryceson, D.M. : Immunological Phenomena in Leprosy and related disorders. Immunol., 13 : 209-266, 1971.

138. Tanphai Chitra, D. : Bulletin of the international union against tuberculosis. 54/2 : 168, 1979.

139. Uberoi, S., Malviya, A.N.; Chattopadhyaya, C. et al : Secondary Immuno-deficiency in milliary tuberculosis. Clin. Expt. Immunol., 22 : 404, 1975.

140. Udani, P.M. and Bhat, U.C. : Tuberculosis of C.N.S. Ind. Paed., 11 : 7, 1974.

141. Udani, P.M. : Incidence of Tuberculosis in children. Ind. J. Child Health, 10 : 515, 1961.

142. Udani, P.M. : Evaluation of tuberculin test in tuberculosis.paper presented at Ist National Conference of Indian Academy of Paediatrics, 1964.

143. Udani, P.M., Parikh, U.C., Shah, P.M. and Naik, P.A. : B.C.G. test in Tuberculosis. Ind. Paed., 8 : 143, 1971.

144. Ustvedt, H.J. : Conference on European BCG Programme. P. 161, Heilmann, London. Quoted by Griep, W.A. and Bleiker, M.A. Tubercle (London), 38 : 259, 1957.

145. Verrier, J., Talwar, G.P. : Immuno-deficiency secondary to other diseases. Progress in immunology, 5 : 366, 1974.

146. Vishwanathan, R. : Quoted by Singh, S. : A clinical study of TBM with a view to assess and correlate its neurological damage. Thesis for M.D. (Paed.), Kanpur University, 1973.

147. Von-Pirquet, C. : Die Kutanae Tuberculin Probe. Med. Klin, 3, 1197, 1907. Quoted by Willis, H.S. and Cummings, M.M. : Diagnostic and experimental methods in tuberculosis. P. 210, 1952. 2nd Ed., Charles C. Thomas, Publishers, U.S.A.

148. Venkata Reddy, M. and Prabhu, T. : Active and total E-rosette forming cells in Pulmonary tuberculosis. Ind. J. Med. Res., 77 : 308, 1983.

149. Wallgren, A. : J.A.M.A., 36 : 702, 1928. Quoted by Dahistron G. and Difs. H. The efficicacy of B.C.G. vaccination, P. 1, 1951.

150. Venkata Reddy, M. and Prabhu, T. : EAC-rosette formating cells in pulmonary tuberculosis. Ind.J. Tub., 32 : 189-191, 1985.

151. W.H.O. Expert Committee on T.B., Eight Report, Geneva, 1964 (WHO Technical Report No. 290).

152. W.H.O. Expert Committee on Biological Standardisation, Eighteenth Report, Geneva, 1966.

153. W.H.O. Expert Committee on Tuberculosis, Ninth Report, Geneva, 1974 (WHO Technical Report No. 552).

154. W.H.O. Tuberculosis Research Office : Bull. of the World Health Organization, 12 : 63, 1955.

155. W.H.O. Tuberculosis Research Office : Bull. of the World Health Organisation, 12 : 85, 1955.

156. W.H.O. Tuberculosis Research Office : Bull. of the World Health Organisation, 12 : 101, 1955.

157. W.H.O. Vaccination against tuberculosis - 6th Report of Expert Committee on Tuberculosis. W.H.O. Technical Report Series, 88 : 1954.

158. Wybran, J., Carr, M.C. and Fundenberg, H.H. : J. Clin. Invest., 51 : 2537, 1972.

159. Wybran, J. and Fundenberg, H.H. : Rosette formation - A test for cellular immunity. Trans. Assoc. Am. Physicians, 34 : 239, 1971.

160. Wybran, J. and Fundenberg, H.H. : Thumus derived rosette formating cells in various human disease Statt. Count Lymphoma, bacterial and viral infection and other diseases. J. Clin. Invest., 52 : 1026, 1973.

161. Yoffey, J.M. : The lymphocyte. Ann. Rev. Med., 15 : 125-131, 1964.

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A P P E N D I X

APPENDIX

DEPARTMENT OF PAEDIATRICS
M.L.B. MEDICAL COLLEGE & HOSPITAL, JHANSI.

TOPIC - IMMUNOLOGICAL PROFILE IN CHILDHOOD TUBERCULOSIS.

WORK SHEET

Investigator : Dr. Ajay Sood.

Guide : Dr. R.S. Sethi,
M.D., D.C.H.,
Lecturer in Paediatrics.

Sl.No. : .... Date : ....

M.R.D./O.P.D. No. : .... Ward/Bed No.: ....

Details of patient :

Patient's name : .... Age/Sex ....

Address : ....

Date of admission : .... Date of discharge : ....

Chief complaints :

(a) Cough :

i) Duration
ii) With or without expectoration
iii) Amount of sputum
iv) Mucoid / Purulent / Mucopurulent
v) Hemoptysis.

(b) Fever :

i) Duration
ii) Severity - mild / high
iii) Type - continuous/intermittent/remittent
iv) Association

(c) Loss of weight :

i) Duration
ii) Amount
iii) Association

(d) Failure to thrive : Yes / No - Duration

(e) Appetite : Lost / Retained
 i) Duration
 ii) Amount

(f) Consciousness : Impaired / Intact

(g) Headache/Vomiting : Yes / No

(h) Convulsion :
 i) Duration
 ii) Type
 iii) Associated factor

(i) Backache :
 i) Duration
 ii) Site
 iii) Discharge

(j) Breathlessness :
 i) Duration
 ii) Onset
 iii) Severity

(k) Chest pain : Present / Absent
 i) Duration
 ii) Site
 iii) Onset
 iv) Relieving factor

(l) Distension of abdomen : Present / Absent
 i) Duration
 ii) Site

(m) Loose motions : Present / Absent
 i) Duration
 ii) Amount
 iii) With or without mucous/blood/both

(n) Alternate constipation and diarrhoea : Present / Absent

(o) Moving ball of gas: Present / Absent
 i) Duration
 ii) Relieving factor

(p) Swelling over neck : Yes / No
i) Duration
ii) Discharge
iii) Pain
iv) Association

(q) Other complaints :

History of past illness :
i) History of measles/exanthem/pertussis
ii) History of diabetes
iii) History of malnutrition
iv) Other

Family History :

Geneological History :

Dietary History :
i) Calories
ii) Protein

Immunisation History :

Socio-economic History :

General Examination :
G.C.
Pulse
B.P.
Icterus
Pallor
Temperature
Oedema
Clubbing
J.V.P.
Lymphadenopathy
Any other -
Hair
Fontanelle
Frontal Bossing

Eyes
Oral cavity
Buccalmucosa
Teeth
Lips
Skin
Subcutaneous
Muscles

<u>Anthropometric examination</u> :

Height
Weight
Head circumference
Mid arm circumference

<u>Systemic Examination</u> :

(a) Respiratory :

i) Inspection
ii) Palpation
iii) Percussion
iv) Auscultation

(b) Cardio-vascular system :

i) Inspection
ii) Palpation
iii) Percussion
iv) Auscultation

<u>Abdomen</u> :

i) Inspection
ii) Palpation
iii) Percussion
iv) Auscultation

<u>C.N.S.</u> :

(a) Special deformity
(b) Higher centres
(c) Cranial nerve examination
(d) Sign of Meningeal irritation

(e) Motor - i) bulk
ii) tone
iii) power
iv) Co-ordination

(f) Sensory

(g) Reflex - i) Superficial
ii) Deep
iii) Planter

(h) Gait

(i) Cerebellar sign

<u>Musculo-skeletal system</u> :

i) Spinal deformity
ii) Any other

<u>Provisional diagnosis</u> :

<u>INVESTIGATIONS</u> :

(a) TLC DLC ESR Hb

(b) X-ray chest / spine / skull

(c) Fundus examination

(d) C.S.F. Examination

(e) Barium follow through

(f) B.C.G.

(g) Mantoux

(h) T. Cell count

(i) B. Cell count

<u>SUMMARY</u> :

<u>COMMENTS</u> :

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